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On *Ancylostoma braziliense* (de Faria, 1910) and its Morphological Differentiation from *A. ceylanicum* (Looss, 1911).

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The species of the genus *Ancylostoma* (Dubini, 1843) reported as parasites of the human intestine are *A. duodenale*, *A. braziliense*, and occasionally *A. caninum* and *A. malayanum*. " Creeping eruption " is considered to be a cutaneous helminthiasis produced by larvae of an ancylostome that American authors accepted as *A. braziliense*. As a result of detailed studies of the parasite by Lane (1922), Darling (1924) and Schwartz (1927), the majority of parasitologists now treat *A. ceylanicum* as a synonym of *A. braziliense*, contrary to the opinions of de Faria (1914, 1916) who described *A. braziliense*, and Looss (1914) who described *A. ceylanicum*. The solution of the problem is not only of systematic but also of epidemiological importance because de Faria (1914) expressed the opinion that *A. braziliense* is not pathogenic for man. In the present enquiry the writer has been able to demonstrate that *A. braziliense* and *A. ceylanicum* are two different species ; and for this reason it is considered of interest to summarise the original descriptions of the two nematodes and the existing opinions concerning this problem.

Historical Review.

In 1910 de Faria recovered from cats and dogs in Brazil an ancylostome that he named *A. braziliense*, giving the following principal characters : female, 8.5 mm. long and 0.35 mm. broad ; male, 7.5 mm. long and 0.22 mm. broad. " The mouth is obliquely projected towards the dorsal aspect . . . The ventral margin of the mouth carries on each side of the middle line a large, strong triangular tooth, the sharp points of which are bent down and backwards. In the superior and internal angle of these teeth a small accessory tooth is to be seen. The dorsal opposite edge has on the middle line an almost semi-circular incision, partly covered by the latter. Two angles project above the

latter. These angles have been described as teeth . . . At the base of the buccal cavity two more short, blade like, triangular teeth, the so-called inner ventral teeth, are to be seen. . . . In the bursa, *with general anatomy as in other species* (present writer's italics) the median lobe is distinctly separated from the lateral lobe. The disposition resembles greatly that of *A. caninum*. The dorsal ray is much longer and the lateral rays are relatively short in comparison to the principal trunk. The ventral rays are as long as in *A. caninum*. The plate 22 dispenses further description. . . . The most common parasite of cats and dogs is *A. caninum* Ercolani, from which our species can be distinguished by the dimensions, number of ventral teeth and caudal bursa. *The latter is not a very secure element for a specific diagnosis.*" (Present writer's italics.)

In 1911 Looss published the description of a new ancylostome collected from a civet cat in Ceylon and he named it *Ancylostoma ceylanicum*. The original description is as follows : " Distinctly smaller than all the preceding species. Male averages 5 mm., female 7 mm., in length. At the anterior edge of the mouth capsule (Fig. 111, Pl. XI) there is, on each side, one large tooth and below, or behind this, on the side turned towards the middle line, a very small tooth of which the tip only looks out from below the large one ; the arrangement of these teeth thus appears to resemble that in the preceding species. The lateral lobes of the bursa (Fig. 120, Pl. XII) are sharply marked off from the dorsal lobe, and *are longest in the direction of the ventral rays*. (Present writer's italics.) All the rays are remarkably thick and plump. No external appendages at the anogenital aperture of the male."

In the original description of *A. braziliense* by de Faria there is no drawing of the lateral view of the bursa, while Looss does not illustrate the dorsal view and ventral view of the bursa in *A. ceylanicum*. Leiper (1913) without having had the opportunity of examining *A. braziliense* but comparing the drawings of the dorsal rays as illustrated by de Faria and Lane, suggested that *A. braziliense* and *A. ceylanicum* were probably one and the same species. After Leiper's publication, de Faria and Looss (1914) again re-examined their material. Looss in a letter to de Faria (1914) wrote,* " *A. braziliense* has only a single tooth on each side which corresponds to the centre one of *A. duodenale*. The outer and inner teeth are completely wanting. *A. ceylanicum* has an inner tooth which I now see to be frequently larger even than that drawn in my *Ancylostoma* publication . . . The length and slenderness

*Translation by Lane (1916) from original in German.

of the bursal rays, especially of the external dorsal ray, is remarkable since in *A. ceylanicum* all the rays are thicker and plumper. The relative thickness of the bursal rays in similar species of *Ancylostoma* is a definite differential character, but this point is emphasised by no recent writer ; on the other hand, one finds full descriptions of the arrangement of the rays, which is *the same for all Ancylostoma* (present writer's italics), and complete details of the terminal divisions of the dorsal ray which vary in nearly every individual . . . In my opinion, therefore, *A. braziliense* and *A. ceylanicum* represent two independent and easily differentiated species."

De Faria (1914), after having received specimens of *A. ceylanicum* collected from dogs of India and sent to him by Lane, concluded that these two worms belong to two different species, the principal difference being in the buccal capsule and in the bursa. According to de Faria, the inner and internal tooth in *A. braziliense* is comparatively much smaller than in *A. ceylanicum* (de Faria did not accept the statement of Looss that the inner tooth of *A. braziliense* is completely missing) and the rays of the bursa, especially the externo-dorsal, in *A. braziliense* are long and slender, whereas in *A. ceylanicum* they are shorter and stouter.

The comparative studies of Looss and de Faria evidently did reveal morphological differences between the two species, but the description of the differences was not at all satisfactory. Leiper (1915) after the publication of de Faria's paper in 1914, reviewed the situation objectively.

Lane (1916) restudied *A. ceylanicum* and stated that : " The ventral rays are stout. The externo-lateral ray is widely separated from the other two lateral rays, whose points lie close together. Opposite to the root of the externo-dorsal ray the dorsal ray has a pronounced ventrad thickening."

In 1922 Gordon recovered 6857 ancylostomes from sixty-seven post mortems performed in Amazonas and found four specimens which were identified as *A. braziliense*. He writes : " There were two males and two females . . . Each worm was found in a separate host. Two were found in a native Amazonian who, so far as is known, had never left the state of Amazonas ; one in a patient who originally came from Ceara, and one in an American of the ' beach comber ' type who had lived some twenty years in North Brazil."

In the same year, 1922, and in the same periodical, Lane published a long systematic study of *A. braziliense* based on specimens of *A. ceylanicum* of his collection and on ancylostomes received from Gordon's

Brazilian collection. Lane's researches induced him to conclude that no evident difference existed between the two species and did not hesitate to treat *A. ceylanicum* as a synonym of *A. braziliense.*

Yorke and Maplestone (1926) accepted the synonymy and modern textbooks on Parasitology and Helminthology also maintain it. Brumpt (1949), in his last edition of Précis de Parasitologie, still treats *A. braziliense* and *A. ceylanicum* as distinct species but does not give detailed descriptions of them.

Personal Researches.

On 7.6.49 the writer collected from the small intestine of a Serval (*Felis serval*) which had died in the Zoological Gardens of Rome approximately two months after its arrival from Somaliland, numerous specimens of an ancylostome with two big ventral teeth, each tooth carrying in the superior and internal angle a very small rudimentary internal tooth. The disposition of the lateral rays in the bursa of this species was completely different from that in *A. ceylanicum* and from those in the other hitherto described species of *Ancylostoma* but similar to that in some species of *Uncinaria.* The comparison of the dorsal view of the bursa of this ancylosome with that recorded for *A. braziliense* by de Faria confirmed its identity with this species. Thus convinced that *A. braziliense* and *A. ceylanicum* were distinct species, a study was made of a large number of ancylostomes collected in different parts of the world, in the collections of the London School of Hygiene and Tropical Medicine, the Liverpool School of Tropical Medicine and in the personal collection of Dr. LeRoux. It is now possible to state with certainty that some of the specimens in these collections exhibited the characters of *A. ceylanicum* as described by Looss and Lane while others, on the contrary, exhibited the characters incompletely described by de Faria and Looss for *A. braziliense,* but well illustrated by de Faria (1910) on Plate 22. These two species can be readily differentiated. Leiper in a recent personal communication stated that he had already observed distinct differences between *A. braziliense* and *A. ceylanicum.*

Table I gives the results of the writer's observations and the distribution of the two species in America, Africa and Asia.

Sample No. 2083 in the London collection consisted of one *A. braziliense* and numerous *A. ceylanicum.* The Brazilian material collected by Gordon from man, dogs and cats, was composed of 35 male *A. ceylanicum* and only one male *A. braziliense.*

The most important differential characters between the two species were evident in the mouth opening and in the bursa as pointed out by de Faria (1914). In *A. braziliense* the inner ventral teeth arising in the bases of the big ventral teeth are generally smaller and more internal. More striking are the differences in the bursa. (*a*) In *A. braziliense* the lateral lobes are clearly longer in the direction of the lateral rays; these rays " are relatively short in comparison to the principal trunk " (de Faria). According to the writer's observations the lateral rays are curved and the medio-lateral ray does not lie close

Table I.

Country of Origin		*Host*	*Species*	*Source and collection number*
America	Br. Guiana	*Canis familiaris*	*A. braziliense*	London No. 2083
	,,	,,	*A. ceylanicum*	,, ,,
	,,	*Felix catus*	,,	,, No. 1352
	Amazonas (Brazil)	*C. familiaris*	,,	L'pool (Gordon)
	,,	*Homo sapiens*	,,	,, ,,
	,,	*F. catus*	,,	,, ,,
	,,	*C. familiaris*	*A. braziliense*	,, ,,
Africa	W. Africa	*C. familiaris*	*A. braziliense*	London No. 1740
	Somaliland	*Felis (Leptailurus) serval*	,,	Ist. Parassit. Roma.
	Transvaal	*C. familiaris*	,,	Dr. LeRoux
	Sierra Leone	*F. catus*	,,	L'pool No. 1001
	Freetown	*Felis pardus*	,,	,, No. 692
Asia	India	*Viverricula malaccensis*	*A. ceylanicum*	London No.44/29
	Berhampore	*F. catus*	,,	L'pool No. 809
	E. India	*Felis bengalensis*	,,	London No. 620/28
	Sumatra	*Felis temminckii*	,,	,, No. 629/28

to the externo-lateral ray. The externo-dorsal rays arise nearer to the origin of the dorsal rays. (*b*) In *A. ceylanicum* the lateral lobes are " longest in the direction of the ventral rays " (Looss 1911); the " ventral rays are stout and the externo-lateral ray is widely separated from the other two lateral rays, whose points lie close together " (Lane, 1916). The externo-dorsal rays arise not so near to the origin of dorsal ray as in *A. braziliense*.

These differences in the bursa of *A. braziliense* and *A. ceylanicum* can be readily appreciated when the lateral views and the dorsal views of these two worms are compared (Figs. 2 B, C and 4 B, C).

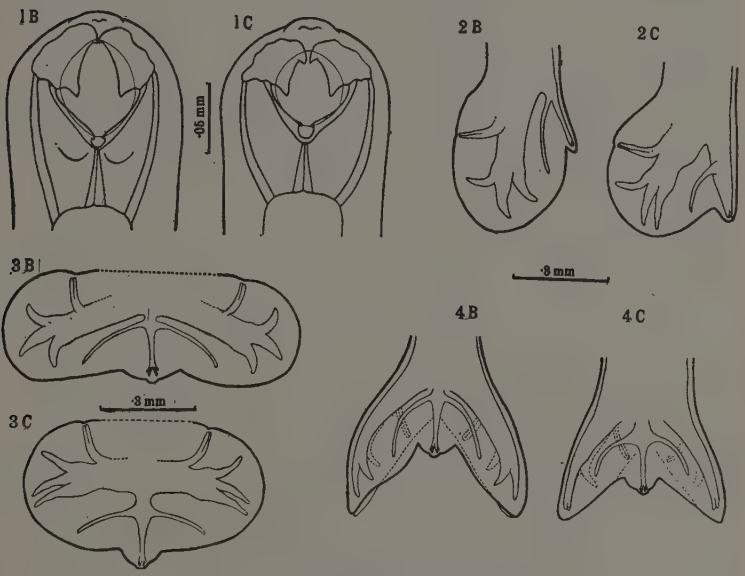
To forestall any suggestion that great individual differences in the disposition of the bursal rays could exist within a species, we have studied numerous specimens from every collection. From sample number 2166 of the London School collection we have carefully examined 50 males and as a result of these observations we can affirm that the disposition of the bursal rays represents a distinct and constant character and that only moderate individual differences occur within the same species. Intermediate forms between *A. braziliense* and *A. ceylanicum*, as regards the disposition of the lateral rays, were never found.

It is the writer's opinion that the present confusion about the validity of these two species must be attributed to the fact that the previous descriptions of *A. braziliense* are somewhat incomplete. De Faria did not record the measurements of the oesophagus, spicules, female tail, etc., and, speaking of the bursa of *A. braziliense*, stated that the general anatomy is as in other species. This is not exactly correct. De Faria did not study the bursa because he believed that it was not a " secure element for a specific diagnosis." Looss also failed to draw attention to the distribution of the rays. He was of the opinion that it is " the same for all *Ancylostoma*." This conception is absolutely incorrect. The critical observations expressed by Lane in 1916 about the inadequate description of *A. braziliense* are indeed well founded.

Through the kindness of Professor Gordon we had the opportunity of studying the ancylostomes which he collected from human post-mortems in Amazonas (1922) and which were used by Lane for his systematic researches on *A. braziliense*. It is thus now possible to indicate the reason for Lane's erroneous conclusions. Those specimens which, probably because of their Brazilian origin, he accepted as *A. braziliense* are in reality typical specimens of *A. ceylanicum*. Evidently any comparison with other *A. ceylanicum* was impossible. Figure 1A of Lane's work (1922), representing the lateral view of a bursa of an ancylostome from Gordon's Brazilian material is evidently not of human origin, but probably came from another collection, perhaps the personal collection of Lane. This figure 1A illustrates for the first time, although in an imperfect manner, the lateral view of the bursa of *A. braziliense*. It is evidently this figure which has created such great confusion and has wrongly convinced the majority of helminthologists that great variations may occur in the disposition of the lobes and rays in the caudal bursa of these worms.

Although it must be admitted that the original description of

A. braziliense as given by de Faria is incomplete and imperfect, yet his illustration of the dorsal view of the bursa is excellent and allows identification of the species. From his illustrations of *A. braziliense*



Ancylostoma braziliense and *A. ceylanicum*.

Fig. 1. Dorsal view of head of *A. braziliense* (B) ; *A. ceylanicum* (C).

Fig. 2. Lateral view of bursa of *A. braziliense* (B) ; *A. ceylanicum* (C).

Fig. 3. Ventral view of opened bursa of *A. braziliense* (B) ; *A. ceylanicum* (C).

Fig. 4. Dorsal view of bursa of *A. braziliense* (B) ; *A. ceylanicum* (C).

it may be concluded that it is not a " species inquirenda." We wish to emend his description of the species as follows :—

A. BRAZILIENSE (DE FARIA, 1910).

The body is whitish, cylindrical, rather slender, attenuated at both extremities in the female, and only anteriorly in the male. The cephalic end is clearly dorsally bent. The cuticle is transversely striated, with striae 4–6 μ apart. The buccal capsule is elongated. In the ventral margin of the mouth there are two dental plates, each carrying

a " large, strong, triangular tooth, the sharp points of which are bent down and backwards. In the superior and internal angle of these teeth a small accessory tooth is to be seen. The dorsal opposite edge has on the middle an almost semi-circular incision " (de Faria). The two anterior angles of this incision, considered as " dorsal teeth " are generally directed caudally. Below the incision appears a thickening of the capsule, the dorsal gutter, through which passes the excretory duct of the dorsal oesophageal gland. " At the base of the buccal cavity, two more short blade-like, triangular teeth, the so-called inner ventral teeth are to be seen " (de Faria). The cuticle of the oral aperture covers the base of the ventral teeth and almost completely covers the dorsal incision. The oesophagus is divided in two parts by the nerve ring, the anterior being clearly shorter and more cylindrical, the posterior longer and swollen. It opens into the intestine with a three lobed valve. Cervical papillae are present but variable in position, sometimes in the first half and sometimes in the second half of the oesophagus. The excretory pore opens on the ventral surface at a point about half way down the oesophagus.

Male. Length : 5.0–7.5 mm. Width : 0.190–0.270 mm. Oesophagus : 0.620–0.750 mm. by 0.150–0.200 mm.

The seminal tubes are slender and not very long and form coils generally not close to one another. The spicules are 0.7–1.0 mm. long, equal, tubular, thin and enlarged in their proximal extremity. The gubernaculum is yellowish brown, 70–80μ long and 16–20μ broad. Prebursal papillae are present at 0.450–0.560 mm. from the lateral extremity of the bursa. The genital cone is present, but not very prominent. The bursa is 0.7–1.1 mm. wide when completely spread. The disposition of the lobes and of the bursal rays resembles that of members of the genus *Uncinaria* more closely than that of the genus *Ancylostoma*. The small dorsal lobe is clearly divided from the lateral lobes which are very long in the direction of the lateral rays. The dorsal ray, generally 0.2 mm. long, is divided into two branches at about 50μ from the posterior extremity. Each of its two branches terminates in two long digitations and a very small third one. The dorsal ray in its second sixth gives off two long and slender externo-dorsal rays.

The lateral rays have a long common origin and are " relatively short in comparison to the principal trunk " (de Faria). The ratio between the length of the rays and the length of the common trunk is approximately 1 : 3. These lateral rays are characteristically curved ; the postero-lateral and medio-lateral are dorsally bent, the externo-

lateral is ventrally bent. They are clearly divergent and the terminal papillae lie distant one from the other. The distance between the terminal papillae of the postero-lateral and medio-lateral rays averages 100μ in the specimens examined and that between the terminal papillae of the medio-lateral and externo-lateral averages 170μ.

The ventral rays have a common origin and run closely parallel till near the edge of the bursa.

Female. Length : 6.5–9.0 mm. Width : 0.220–0.320 mm. Oesophagus : 0.780–0.820 mm. long by 0.170–0.220 mm. broad. The genital tubes are not very long and relatively stout. The vaginal opening is generally at the junction of the medial third with the posterior third of the body. The anus is at 0.150–0.240 mm. from the caudal extremity. The last part of the body is irregularly conical and generally terminates in an acute point.

Discussion.

The demonstration of the validity of the species *A. braziliense* and *A. ceylanicum* creates new epidemiological problems. In fact, there is now no proof that man is a host of the adult of *A. braziliense*, while *A. ceylanicum* is, in some parts of the world, a relatively frequent intestinal parasite of man. Similarly, there is considerable doubt as to whether " creeping eruption " in man has ever been produced by the larvae of *A. braziliense*. Unfortunately, Shelmire (1928), Dove (1932), etc., do not furnish us with any data as to the exact identity of the larvae with which they reproduced the creeping eruption in volunteers. None of the Americans who have studied this problem gives a description of the adult worm and all seem to accept *A. ceylanicum* and *A. gilsoni* as synonyms of *A. braziliense* ; whereas, in reality, *A. gilsoni* is a synonym of *A. ceylanicum* which has been demonstrated to be completely different from *A. braziliense*. It now remains to be proved whether or not *A. braziliense* is pathogenic for man either in the adult or larval stage.

Summary.

After a detailed study of numerous ancylostomes in the collection of the London School of Hygiene and Tropical Medicine, the Liverpool School of Tropical Medicine and the personal collection of Dr. LeRoux, it has been possible to demonstrate that the names *A. braziliense* (de Faria, 1910) and *A. ceylanicum* (Looss, 1911) are not synonymous as has been accepted by the majority of parasitologists, but the names of two different species. A new amended description of *A. braziliense* is given.

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The writer wishes to express his gratitude to Dr. P. L. LeRoux and Professor J. J. C. Buckley for their constant help during these researches.

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On *Ancylostoma paraduodenale*, a New Species from Felines, Closely Related to *A. duodenale*.

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In 1911 Looss wrote as follows : " Up to the present time *Agchylostoma duodenale* is known to occur with certainty only in Man. It is true that the older works and text-books state that it has also been found in various species of animals (some species of apes, the tiger, the dog, the horse, etc.). In opposition to these statements, attempts made on various occasions to infect adult dogs, cats, rabbits, guineapigs, rats, mice, etc., with the parasite yielded entirely negative results. Only with quite young dogs and cats (not more than three or four months old) was the infection partly successful. The results of all these attempts at artificial infection thus support the view that the older statements as to the natural occurrence of *Agchylostoma duodenale* in animals are founded on a confusion of this species with others . . ." Since Looss's publication, *A. duodenale* has again been reported several times from non-human hosts which were listed in 1936 by van der Berghe. But the accuracy of these records is open to some doubt since they are either accompanied by no description of the parasite or by an inadequate one. In view of the possible occurrence in animals of ancylostomes closely resembling *A. duodenale*, such as the species about to be described, it is all the more necessary now that all claims to have found *A. duodenale* in a non-human host should be substantiated by a very detailed description of the specimens. The matter is moreover of some importance epidemiologically as the question of a reservoir host for *A. duodenale* is involved.

Ancylostoma paraduodenale n.sp., from the Serval.

From the small intestine of two Servals, *Felis* (*Leptailurus*) *serval*, which died in the Zoological Gardens, Rome, on 7.6.1949 and on 17.6. 1949, approximately two months after their arrival from Somaliland, there were collected 5 specimens (2 males and 3 females) and 17 specimens (10 males and 7 females) respectively of an *Ancylostoma* sp. which is in some respects closely related to *A. duodenale*. Having in mind the possibility of the Serval acting as reservoir of a parasite

pathogenic to man, all the specimens were carefully examined and found to be, not *A. duodenale*, but a new species which, on account of its affinities, we propose to name *Ancylostoma paraduodenale* n.sp.

In a collection of helminths made by Dr. LeRoux in Northern Rhodesia, it was observed by the writer that *A. paraduodenale* also occurs in *Felis leo*, *Felis caffra* and *Acinonyx jubatus*. It is of interest to note that *A. duodenale* has not been reported from man in this part of Africa.

Morphology (Figs. 1–7).

The body is whitish, rather slender, the cephalic extremity dorsally bent. The cuticle is striated transversely with striae 4.2–6.8μ apart. The buccal capsule (Figs. 1, 2 and 3) has the shape of an elongated cup. Ventrally there are two dental plates each carrying two prominent teeth, the external being a little stouter than the internal. The inner tooth generally has near its internal base a tubercle, sometimes in the form of a rudimentary or very small third tooth. It was observed that there is considerable variation in the size of this third tooth. In some specimens it is practically missing, in others rudimentary and in others it is quite prominent. The " root " of the teeth gives to the outer border of the capsule an irregular appearance. The dorsal edge of the buccal capsule shows an incision which is clearly less deep than in *A. duodenale* and is not U-shaped, but is strongly curved so that the maximum diameter is in the central part of the incision (Figs. 8 and 9). The so-called dorsal teeth appear as small tubercles which do not project clearly beyond the cuticle covering the oral capsule.

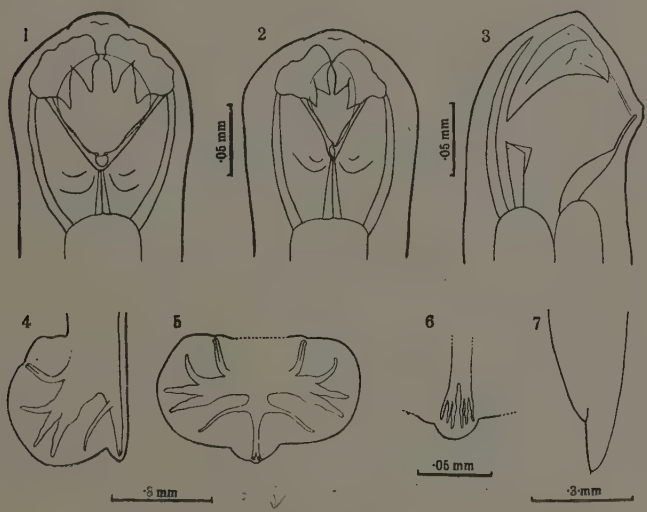
Below the dorsal incision appears a thickening of the capsule, the so-called " dorsal ridge," perforated by the duct of the dorso-oesophageal gland. In the base of the buccal cavity are the two triangular subventral lancets as seen in the other species of *Ancylostoma*. The cuticle overlaps the base of the ventral teeth and covers the dorsal teeth almost completely.

The oesophagus is swollen in the posterior part and its opening into the intestine is furnished with the usual lobed valve. The nerve ring surrounds the oesophagus in the second part of its anterior half. The cervical papillae are well developed, conical and may be situated either anterior or posterior to the middle of the oesophagus. The excretory pore opens generally at the level of the middle of the oesophagus.

Male. Length : 5–8 mm. (average 6.8 mm.). Maximum width : 0.210–0.240 mm. (average 0.230 mm.). The oesophagus is 0.620–0.730 mm. long (average 0.656 mm.). The nerve ring divides the

oesophagus into two parts, the anterior being slightly shorter and cylindrical in shape and the posterior with the greater diameter. The vesicula seminalis is placed generally, but not always, in the second half of the medial third of the body.

The genital tubes are long and slender and closely coiled. The spicules are 1.10–1.50 mm. long (average 1.25 mm.), equal, tubular, thin and delicate, enlarged in their proximal extremity. The gubernaculum is approximately 80μ long by 20μ broad, angular in shape and



Ancylostoma paraduodenale n.sp.

Fig. 1. Head, dorsal view (opened mouth). Fig. 2. Head, dorsal view (closed mouth). Fig. 3. Head, lateral view. Fig. 4. Bursa, lateral view. Fig. 5. Bursa, ventral view. Fig. 6. Dorsal ray. Fig. 7. Posterior extremity of female, lateral view.

enlarged in the posterior tract. Prebursal papillae are present. The bursa (Figs. 4 and 5) in maximum width, when spread, ranges from 0.53 mm. to 0.7 mm. The general disposition of the lobes and of the rays is similar to that of *A. duodenale*. The dorsal lobe is small, three lobed, not deeply notched but clearly divided from the others. The dorsal ray is nearly 0.2 mm. long, and gives origin to two branches at about 0·03–0·04 mm. from the posterior end. These two branches are generally not very divergent and each branch is tridigitate. (Fig. 6.)

The externo-dorsal rays arise in the second proximal sixth of the dorsal ray and are rather long. The three lateral rays have a common stem. The postero-lateral and medio-lateral rays are straight and slightly divergent while the externo-lateral diverges clearly from the others and is rather long.

The space dividing the postero-lateral and the medio-lateral rays is a little deeper than that dividing the medio-lateral and externo-lateral, but generally not so deep as in *A. caninum* and *A. duodenale*. The relation between the distance of the postero-lateral and medio-lateral terminal papillae and the distance of the medio-lateral and externo-lateral terminal papillae ranges from 1 : 1.6–1 : 2.7 (average in 12 specimens 1 : 2). The two ventral rays are relatively long, arise from a common stem and run parallel and close together till near the edge of the bursa. The ventro-ventral ray is generally a little longer than the postero-ventral and the extremities are slightly curved caudally.

Female. Length : 6.5–8.5 mm. (average 7.7 mm.). Width : 0.26–0.315 mm. (average 0.29 mm.). Oesophagus : 0.63–0.78 mm. long (average 0.726 mm.). The genital tubes are long and slender, forming numerous closely arranged coils. The vaginal opening is in the middle third, near to the posterior third of the body. The anus opens from 0.15–0.18 mm. from the caudal point. The tail is irregularly conical, flattened dorso-ventrally and terminates in an acute point (Fig. 7).
Eggs : similar to those of *A. duodenale*, average $35\mu \times 55\mu$.

Host : Felis serval.

Habitat : Small intestine.

Type locality : Somaliland.

Type specimens in the helminthological collection of the British Museum ; co-types in the collection of the Department of Parasitology, London School of Hygiene and Tropical Medicine and in the Istituto di Parassitologia, Rome.

Relationships of Species of *Ancylostoma*

The species of the genus *Ancylostoma* are listed by Yorke and Maplestone (1926) as follows :

A. duodenale (Dubini, 1843) type species of the genus.
A. braziliense (de Faria, 1910) (Syn. *A. ceylanicum* (Looss, 1911)).
A. caninum (Ercolani, 1859).
A. conepati (Solanet, 1911).
A. gilsoni Gedoelst, 1917.
A. malayanum (Alessandrini, 1905).
A. minimum (Linstow, 1906).

A. mucronatum (Molin, 1861).

A. mycetis nom. nov., Yorke and Maplestone, 1926 (Syn. *Diploodon quadridentatum* Molin, 1861).

A. pluridentatum (Alessandrini, 1905).

Since 1926, the following species have been described : *A. mephitis* Micheletti, 1929 ; *A. martinagliai* Mönnig, 1931 ; *A. duodenale galagoi* van der Berghe, 1936 ; *A. hescheleri* Mönnig, 1938 and *A. caninum* var. *longispiculata* Mönnig, 1938.

Some of these species are briefly described and difficult to identify. *A. mycetis* was described by Molin (1861) who had only one female specimen. Schwartz (1922) is of the opinion that *A. mucronatum* is probably the same as *A. conepati* (Solanet, 1911). *A. minimum* is inadequately described. The morphological characters of *A. gilsoni* are no different from those given by Looss (1911) and Lane (1913) for *A. ceylanicum*. *A. duodenale galagoi*, considered by van der Berghe (1936) as subspecies of *A. duodenale* is stated to have a three-toothed ventral plate, and there is no description of the male bursa. The number of valid species therefore appears to be less than that listed by Yorke and Maplestone (1926).

That *A. ceylanicum* (Looss, 1911) is a synonym of *A. braziliense* (de Faria, 1910) cannot be accepted. Lane (1922) expressed the opinion that these two names were synonymous and this was accepted by the majority of helminthologists. But it has been demonstrated by the writer in a recent paper (1951) that these two species can easily be differentiated by the structure of the oral capsule and still more readily by the disposition of the rays of the bursa. In *A. braziliense* the disposition of the bursal rays is completely different from that of other known species of the genus *Ancylostoma* and closely related to the type met with in the genus *Uncinaria*.

An attempt to divide the genus *Ancylostoma* into two subgenera, *Ancylostoma* and *Ceylancylostoma*, was made by Lane (1916) on the basis of the number of ventral teeth in the buccal capsule and the disposition of the lateral rays in the bursa.

These he defined as follows : " In the one group, that of the three-toothed forms, the medio-lateral and dorso-lateral rays are separated by a deep cleft (considerably deeper than that between the externo-lateral and medio-lateral), these rays are divergent and their terminal papillae lie at considerable distance from one another. In the other group, that of the two-toothed forms, the two clefts between the three lateral rays are of much the same depth, while the medio-lateral

and the dorso-lateral rays lie side by side with their terminal papillae in close proximity. It is obvious that with the three-toothed type of mouth armature there is associated one type of arrangements of the bursal rays, and with the two-toothed type another." According to Lane the subgenus *Ancylostoma* was to accommodate the three-toothed species (or two-toothed with a rudimentary third one as in *A. duodenale*) while the subgenus *Ceylancylostoma* was for the two-toothed species which, when Lane published his work were *A. malayanum*, *A. ceylanicum* and *A. braziliense*; *A. gilsoni*, *A. mephitis* and *A. hescheleri* have been described since. The disposition of the lateral rays of *A. pluridentatum* is of the *Ceylancylostoma* type, while that of *A. martinagliai* is of the *Ancylostoma* type.

Another attempt to classify the species of the genus *Ancylostoma* was made by Micheletti (1929) who considered of diagnostic value the level at which the externo-dorsal ray arises from the dorsal ray.

Differential Characters of *A. paraduodenale* and *A. duodenale*

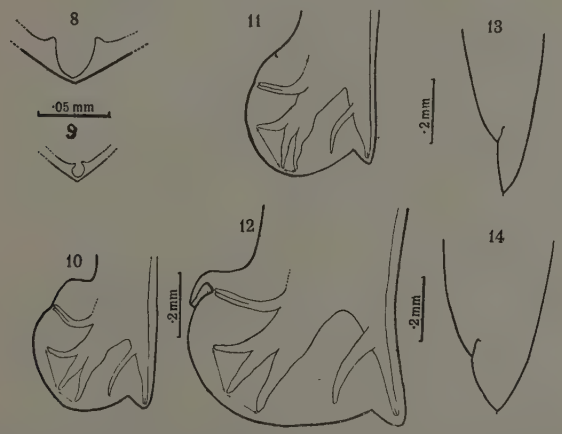
The only species with which *A. paraduodenale* may be confused is *A. duodenale*. For this reason we have compared it with several specimens of *A. duodenale* from man in different parts of the world and we have been able to demonstrate the following differences:

(*a*) *Dimensions and shape of the worms: A. paraduodenale* is smaller than *A. duodenale* in length and width and the head seems to be more dorsally bent. We consider these not very important differential characters since it is known that the same worm in different hosts can assume different proportions and because the degree of the dorsal curvature of the head can be influenced by methods of fixation.

(*b*) *Mouth opening:* ventrally, the third ventral inner tooth in *A. duodenale* is constantly a small and rudimentary one, while in *A. paraduodenale* it presents remarkable variations of size and shape, being sometimes apparently absent, sometimes merely a very small tubercle, and sometimes, but rarely, appearing as a small tooth. Dorsally, the incision in the dorsal edge of the mouth capsule is in *A. duodenale* deeper and more U-shaped with the limbs of the U only slightly curved; the "dorsal teeth" project forward beyond the cuticle of the mouth opening. The same incision in *A. paraduodenale* is smaller with an irregularly rounded shape; the "dorsal teeth" are similar to two tubercules and are covered almost entirely by the cuticle. (Figs. 8 and 9.)

(*c*) *Male bursa:* The bursa of *A. duodenale* has the characters described by Lane for the subgenus *Ancylostoma*, i.e. "the medio-lateral

and dorso-lateral rays are separated by a deep cleft (considerably deeper than that between the externo-lateral and medio-lateral); these rays are divergent and their terminal papillae lie at considerable distance from one another." In *A. paraduodenale* the cleft separating the dorso-lateral and medio-lateral rays is not so deep. These two rays are less divergent and the externo-lateral ray is more divergent and longer than in *A. duodenale*. By examining several specimens of *A. duodenale* and *A. ceylanicum* it was observed that the ratio of the distance between the terminal papillae of the mediolateral-postero-lateral rays and the mediolateral-externolateral rays is, on an average, 1 : 1.3 in *A. duodenale* and 1 : 3.5 in *A. ceylanicum*. In *A. paraduodenale* the average in 12 males was 1 : 2 (Figs. 10, 11 and 12).



Ancylostoma paraduodenale n.sp. compared with *A. duodenale* and *A. ceylanicum*. Fig. 8. *A. duodenale*, dorsal incision in buccal capsule. Fig. 9. *A. paraduodenale*, dorsal incision in buccal capsule. Fig. 10. *A. paraduodenale*, lateral view of bursa. Fig. 11. *A. ceylanicum*, lateral view of bursa. Fig. 12. *A. duodenale*, lateral view of bursa. Fig. 13. *A. paraduodenale*, lateral view of female tail. Fig. 14. *A. duodenale*, lateral view of female tail.

(*d*) *Female tail:* The tail of the female of *A. duodenale* is conical, short and not flattened dorso-ventrally, while in *A. paraduodenale* it is irregularly conical and distinctly flattened dorso-ventrally (Figs. 13 and 14). None of the other species of *Ancylostoma* nor the subspecies, *A. duodenale galagoi*, can be confused with this new species.

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*On Two Helminths from the Orang Utan, *Leipertrema rewelli* n.g., n.sp. and *Dirofilaria immitis* (Leidy, 1856).

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This paper comprises descriptions of two helminths, *Leipertrema rewelli* n.g., n.sp. and *Dirofilaria immitis* from the Orang Utan, a rare animal which is confined to the Malaysian sub-region. Also recorded from the same host are *Ascaris lumbricoides* Linnaeus, 1758, *Trichuris trichiura* (Linnaeus, 1771) and *Enterobius buckleyi* Sandosham, 1950.

Leipertrema rewelli n.g., n.sp., a new Dicrocoelid Trematode from the Orang Utan.

The material studied consists of twenty-two mature specimens collected by Dr. R. E. Rewell of the London Zoological Gardens from the pancreas of an Orang Utan (*Pongo pygmaeus*) from Borneo, which had died soon after its arrival in London. The writer wishes to acknowledge his indebtedness to Professor J. J. C. Buckley, under whose supervision the work was done, for the opportunity of examining and reporting upon the material and, through Professor R. T. Leiper, F.R.S., to the Commonwealth Bureau of Agricultural Parasitology (Helminthology) at St. Albans for helpful criticisms and assistance with the literature. He is also grateful to Dr. P. L. LeRoux for his assistance in the study of these specimens and also of the *Dirofilaria* material.

* Part of a thesis approved by the University of London for the award of the Ph.D. degree.

Morphology (Fig. 1).

These flukes are medium sized and leaf-like with a delicate and translucent body. They are elongate with a smooth or non-denticulate outline, the maximum width being at about the junction of the anterior and middle thirds. They measure from 2.5 to 3 mm. in length and 0.7 to 0.9 mm. in maximum width. The cuticle is covered with scattered minute tubercle-like spines which readily tend to become detached. The musculature is poorly developed.

The mouth is sub-terminal. The oral sucker measures 0.16 to 0.18 mm. in diameter and is slightly smaller and less muscular than the ventral sucker which has a diameter of 0.2 to 0.24 mm. The ventral sucker is situated at about the junction of the first and second quarters of the body. The mouth opens into a small muscular pharynx about 0.08 mm. in diameter. The narrow thin-walled oesophagus divides into the intestinal caeca dorsal to the cirrus sac or the anterior portion of the ventral sucker. The gut branches which are simple, diverge slightly from the point of origin and run dorsal to and, in the main, median to the testes. In most of the specimens the gut branches become pouch-like distally. The caeca terminate just beyond the level of the testes about 1.0 to 1.25 mm. from the anterior extremity.

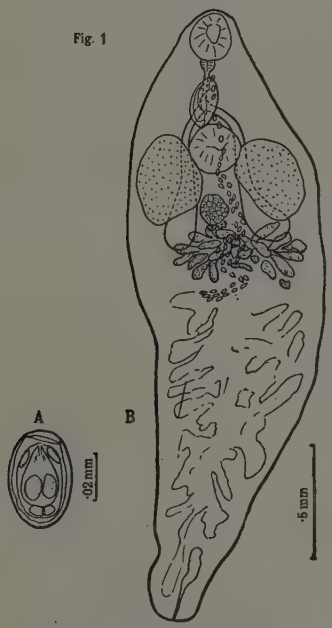
The genital pore is in the median line just behind the level of the pharynx. The oval cirrus sac is prominent and lies in the median plane in front of the ventral sucker. The muscular cirrus is protrusible.

The testes are large and compact rounded masses lying side by side and occupying the area of the body at about the junction of the anterior and middle thirds. They are separated from one another and the anterior space between them is occupied by the posterior portion of the ventral sucker. In some specimens the testes are somewhat oval with the long axis antero-posteriorly. In a few, they appear to be slightly lobulated probably as a result of pressure during examination.

The ovary is small and round, and about 0.13 mm. in diameter. It is situated in the mid-line or slightly to one side at the level of the posterior border of the testes. Immediately behind it is the shell gland. The uterus, containing numerous eggs, consists of a descending and ascending tube with transverse coils in the region between the ovary and the posterior end of the body. In front of the ovary, the uterus takes a relatively straight course to the genital atrium. The eggs are operculated and have thick yellowish-brown shells. They measure 39 to 46μ by 22 to 30μ. Those in the uterus near the genital pore contain almost fully-formed miracidia. The vitelline glands are

composed of about 14 to 20 elongate oval masses which, instead of forming two widely separated groups one on each side of the body, tend to unite towards the mid-line. Where this is complete, they appear almost like a band extending across the middle of the body.

The excretory pore opens at the posterior extremity in the mid-line. The terminal portion of the excretory canal is narrow but it soon dilates to form a wide bladder lying dorsal to the uterine mass and extending for a distance of 0.3 to 0.4 mm. from the posterior extremity.



Leipertrema rewelli n.g., n.sp.
Fig. 1. A. Egg. B. Ventral view of adult.

Relationships.

Of the several genera grouped under the Dicrocoeliidae, the specimens here described approach most closely to *Concinnum* Bhalerao, 1936, *Eurytrema* Looss, 1907, *Platynosomum* Looss, 1907, and *Brodenia* Gedoelst, 1918.

Members of the genus *Brodenia* have a prominent denticulate enlargement about the middle of the body and the testes are contiguous. In *B. serrata* Gedoelst, 1918, obtained from *Cercocebus* sp. in the Belgian Congo, the vitellaria occupy the lateral fields, the caeca extend to about the middle of the body and the cuticle is said to be smooth. Also, a marked dorsi-flexion of the anterior end is described in *B. serrata*. *B. lacinata* (de Blainville, 1820) from the Mandrill (*Papio sphinx*) is a much larger parasite measuring 13.5 mm. in length, with fewer but better developed lateral denticulations than in *B. serrata*.

In *Platynosomum* Looss, 1907, which inhabits the liver and intestines mostly of birds and members of the cat tribe, the intestinal caeca are relatively long and external to the testes. The vitelline glands are confined to the lateral fields.

In *Eurytrema* Looss, 1907, which is found in the pancreas of ruminants, the body is broad, the testes are far apart, the intestinal caeca are relatively long and lateral to the testes, the vitelline glands are extra-caecal in position, the genital pore opens behind the point of bifurcation of gut and the cirrus sac extends backwards beyond the anterior margin of the ventral sucker.

Bhalerao (1936) split the genus *Eurytrema* Looss, 1907, into a number of subgenera including *Concinnum* which Travassos (1944) raised to generic rank. In this genus, the gut branches are lateral to the testes and extend to one-third to one-quarter of the body length from the posterior extremity. The transverse coils of the uterus occur in front of the ventral sucker.

Although Travassos (1944) has shown that considerable variation exists in the individual species of the Dicrocoeliidae, the fluke here described differs in certain important respects from the members of the known genera of the family, viz., the gut branches are very short and lie dorsal to the testes which extend outwards considerably beyond the caeca. The vitellaria tend to be clumped and to extend inwards to meet one another. For these reasons it is proposed to erect a new genus *Leipertrema* for this species which is now named *L. rewelli* n.sp., after Dr. R. E. Rewell who collected the material.

Leipertrema n.g.

Generic diagnosis: Dicrocoeliinae : Body lanceolate with a smooth outline, broadest in front of the middle. Testes rounded, side by side (not contiguous), immediately behind ventral sucker. Intestinal caeca short, reaching a level which is less than half the length of the body from the anterior extremity. The gut branches are dorsal and, in the main,

medial to the testes. Vitellaria posterior to the testes, the two lateral masses tending to meet towards the mid-line. Transverse coils of uterus confined to area behind the ventral sucker. Parasitic in pancreas of Primates.

Type species : L. rewelli n.sp. with the characters described above.

Family : Dicrocoeliidae Odhner, 1911.
Genus : Leipertrema n.g.
Species : L. rewelli n.sp.
Host : Pongo pygmaeus.
Location : Pancreas.
Locality : Borneo.

Type slide deposited in the Helminthological Collection of the London School of Hygiene and Tropical Medicine.

Dirofilaria immitis (Leidy, 1856), from the Orang Utan.

Vogel and Vogelsang (1930) described two filarial worms from the Orang Utan (*Pongo pygmaeus*). One species consisting of two males only was obtained from the heart and named *Dirofilaria pongoi* ; the other was from the muscles and being an incomplete female was not identified. The specimens here described consist of a male and a female said to have been found lying free over the liver in the peritoneal cavity of an Orang Utan which had died in this laboratory. The known species of *Dirofilaria* fall into two main groups, those inhabiting the heart and the main vessels (subgenus *Dirofilaria* Faust, 1937) and those in the tissues (subgenus *Nochtiella* Faust, 1937). The structure of the worms here described shows that they belong to the former subgenus. During the post-mortem examination the thoracic organs had been examined before the abdominal organs and since the worms were found lying free in the peritoneal cavity, it is probable that in reality they are from the heart or the main vessels.

Morphology (Fig. 2).

The worms are white and filiform with a thick cuticle and faint transverse striations. The anterior end is conical with a bluntly rounded tip. There are ten pairs of cephalic papillae, one pair being lateral and the rest sub-medial in position.

Male : The total length of the male worm is 117 mm. and its greatest diameter is 0.57 mm. The diameter of the head is 0.37 mm. The posterior end is attenuate and exhibits a corkscrew coiling through two-and-a-half spirals. The oesophagus is 1.6 mm. in length and as

in the female, there is no noticeable subdivision of oesophagus into anterior and posterior parts. The tail measures 0.12 mm. and the caudal alae extend anteriorly on each side for a distance of about 0.4 mm. from the posterior extremity. The maximum breadth of the caudal alae is 60 microns.

There are four pairs of large symmetrically arranged pre-anal caudal papillae. The spicules in this specimen are partially extruded making it somewhat difficult to be definite about the arrangement of the papillae around the cloacal aperture. There is a slightly thickened transverse band immediately in front of the cloacal opening from which arises a papilla very near the median line. Viewed from certain angles this papilla appears to be double. There is a pair of small adanal papillae and six pairs of post-anal papillae. Of the six post-anal pairs, the first is small and sub-medially placed, the second relatively large and lateral in position, the third and fourth lateral and filiform, the fifth small and sessile and sub-median while the last pair is large and sessile.

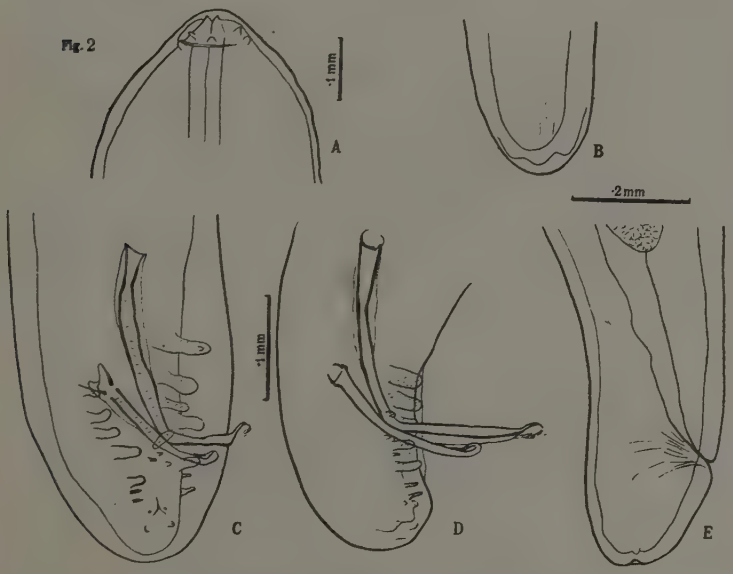
The spicules are unequal and dissimilar. The left spicule (the larger one) measures 0.344 mm. in length and has an elbow-like bend about the middle. Distally, it tapers into a curved filiform portion to which is attached a membrane which clothes the dorsal surface of the spicule. The right spicule is spatulate and gently curved. It measures 0.185 mm., giving a ratio of spicule of 1 : 1.85. $\alpha = 205$; $\beta = 73$; $\gamma = 975$.

Female: The female is larger than the male, the total length being 140 mm. and the greatest diameter 0.8 mm. The diameter of the head is 0.44 mm. The oesophagus is 1.63 mm. long and the nerve ring is situated 0.5 mm. behind the anterior end. The vulva is 3.8 mm. from the anterior end and the uterus is filled with rounded bodies about $25 \times 15\mu$ in size. No microfilariae are present in the uteri. The tail is 0.15 mm. long and is somewhat dorsally directed. The tip of the tail is not smoothly rounded owing to the presence of what appear to be two large blunt papillae. Seen from the dorsal side the tip of the tail presents a trilobed appearance. $\alpha = 138$; $\beta = 147$; $\gamma = 1{,}600$.

Relationships

There seems to be little doubt that this is identical with the male described by Vogel and Vogelsang (1930) as *D. pongoi* from the heart of an Orang Utan and it is probable that the membrane was not evident in their specimens as the spicules were not extruded. Their drawing suggests that a pre-anal median papilla was present although they make no mention of it in their description.

The specimens here described were compared with specimens of *D. immitis* (Leidy, 1856) from dogs from Malaya and no clear-cut morphological differences were found by which they can be distinguished. The examination of literature shows that *D. immitis* is very variable in size and proportions and in none of the systematically important morphological features are the differences sufficiently great or constant for this species from the Orang Utan to be considered a different



Dirofilaria immitis from Orang Utan.

Fig. 2. A. Lateral view of head end. B. Ventral view of tip of female tail. C. Ventro-lateral view of male tail. D. Lateral view of male tail. E. Lateral view of female tail.

species. The practice of accepting forms from different types of hosts specifically distinct is not justifiable especially in the case of worms carried by blood-sucking insects, unless the vector is known to show restricted host preferences.

It is therefore maintained that these specimens from the Orang Utan are *D. immitis* (Leidy, 1856), and that *D. pongoi* Vogel and Vogelsang, 1930, should be sunk as a synonym of the former.

SUMMARY.

1. A new Dicrocoelid trematode, *Leipertrema rewelli* n.g., n.sp., from the pancreas of an Orang Utan which died in the London Zoological Gardens, is described, and the relationships to other genera are discussed.

2. A male and female *Dirofilaria* from the peritoneal cavity of the Orang Utan are described and identified as *D. immitis* (Leidy, 1856).

3. Reasons are advanced for the view that *D. pongoi* Vogel and Vogelsang, 1930, is a synonym of *D. immitis*.

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Journal of Helminthology, Vol. XXV *Nos.* 1/2, 1951. *pp.* 27—28.

Common Vetch, *Vicia sativa* L., as a Host of the Oat Strain of Stem Eelworm *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936.

By L. N. Staniland, A.R.C.S., D.I.C. and J. F. Southey, M.A.
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During March, 1950, the writers visited a 15-acre field of mixed oats and vetches near Lechlade, Gloucestershire, because it was reported that the crop was patchy and that both oats and vetches were diseased. Inspection of the field revealed typical " tulip-root " symptoms in the oats, while many of the vetches were severely dwarfed and showed browning of the lower parts of the stems. Subsequent examination of plants in the laboratory showed the presence of large numbers of *Ditylenchus dipsaci* in the oats and a rather lighter infestation in the vetches ; in the latter the eelworms seemed to be associated particularly with the necrotic tissues in the lower parts of the stems. It may also be mentioned that young docks, probably *Rumex crispus* L., from the same field were found to contain *D. dipsaci*. The host list of plant nematodes, Goodey (1940), gives *Vicia sativa* L., as having been recorded by Bos in 1906, as a host of *Anguillulina* (*Ditylenchus*) *dipsaci*. Dr. T. Goodey has, however, traced the original paper and has informed the writers that it referred to *Vicia villosa* Roth., and not to *Vicia sativa*. Goodey discusses fully in a separate paper the matter of records of *D. dipsaci* in the two *Vicia* species.

Since the infested oats and vetches were present in the same patches it was surmised that both might contain the same strain of *D. dipsaci*. It was therefore decided to set up cross-infestation experiments in order to find out if the eelworms could pass freely from oats to vetches and vice versa. This was carried out as follows.

A sample of the mixed oats and vetch seeds as used in the field where the attack occurred was obtained from the farmer. The two kinds of seeds were separated and treated with a solution of 1 : 4000 iodine in potassium iodide for 10 minutes to eliminate any possibility of seed-borne infestation (Staniland, 1950). This was carried out in large funnels to which were attached rubber tubes closed by pinch-cocks, the seeds afterwards being washed by passing clean water through the funnels.

Two flower-pots were prepared with partially sterilised soil to which was added some peat and a little sand. Some of the infested vetch plants were air-dried, broken up and then mixed into the soil

in one pot ; some " tulip-root " oat material, similarly prepared, was added to the other. Some of the oat seeds were sown in the pot inoculated with vetch material and the vetch seeds in the pot inoculated with " tulip-rooted " oats ; the date of sowing was 5th April, 1950.

After three weeks the oat seedlings were showing marked " tulip-root " symptoms while the vetch seedlings were slightly stunted and distorted. Sample seedlings were removed from each pot, washed and teased up in water. Microscopic examination showed that large numbers of *D. dipsaci* were present in the oats and smaller numbers in the vetches. A week later the symptoms in the vetches were more marked and typical specimens are shown in the accompanying photograph (Fig. 1). The main symptoms were severe stunting and shortening of internodes, together with some twisting and distortion of the leaves. Little or no swelling of the stems was apparent, but the lower parts showed brown necrotic streaks, which were also observed on the plants in the field. In these, also, the eelworms were found to be associated with these brown areas.

Many of the less badly attacked vetch plants seemed to grow away from the trouble later on and produced fairly normal flowers and seeds. Some of the seed was gathered in August and soaked in a Baermann funnel to see whether any infestation had reached it ; however, no eelworms could be found.

From the foregoing it is evident that the common fodder vetch, *Vicia sativa* L., must now be added to the list of plants susceptible to the oat strain of *Ditylenchus dipsaci*, and sowing of this should therefore be avoided in fields known to be infested with this eelworm.

In his paper Goodey discusses the possible relationships between the oat strain and the rye strain found on the Continent. In this connection it may be of interest to record that there was a piece of rye immediately adjoining the oats and vetches in the field at Lechlade. In his original letter about the case, the District Advisory Officer mentioned that although in general the rye was healthy and the trouble ended at the junction of oats and rye, in some places it was beginning to appear in the rye also. Unfortunately, the rye was not examined at the time of the visit, since the possibility of its being also affected by the eelworms was not considered.

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Fig. 1. Photograph of plants of *Vicia sativa* L. experimentally infested with *Ditylenchus dipsaci* from oats, showing two badly affected plants and one from the same pot which remained fairly healthy.

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Stem Eelworm Attack on Seedlings of Vetches, *Vicia villosa* Roth. and *Vicia sativa* L.

By T. Goodey, O.B.E., D.Sc., F.R.S.
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The finding of tares or common vetch, *Vicia sativa* L., attacked by the stem eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev, reported in the foregoing paper by Staniland and Southey and the correspondence on the subject which passed between Staniland and Goodey, brought to light an interesting point in regard to the accuracy of the first record of the parasite on this particular host which calls for some comment and elucidation. Further to the same matter, it was agreed between Staniland and Goodey that it would be valuable if the latter carried out some pot experiments to test the susceptibility of both *Vicia sativa* and *V. villosa* to attack by the oat race of the parasite since, as will be shown presently, *V. villosa* was the species of vetch first found to be infested with the eelworm and not *V. sativa*. It will be convenient, in the present communication, to deal first with the matter of the host records and then with the experimental results.

Host Records.

In the writer's host lists, see Goodey (1929) and (1940), the citation of Bos (1906) as authority for *Vicia sativa* L. as a host of the stem eelworm is incorrect. It should have been Bos (1917), in which a list of plants parasitized by the stem eelworm is given with the following entry on p. 122: "*Vicia sativa* (voederwikke)." There is a footnote reference to the *Tijdschrift over Plantenziekten*, Vol. 12, p. 93, 1906, as authority for the original record. On consulting the earlier volume of the *Tijdschrift over Plantenziekten*, one finds on p. 93 a paper by H. Mayer Gmelin entitled "Over het voorkomen van *Tylenchus devastatrix* in Lupinen en de daarint voor de landbouwpraktijk de trekken conclusies." On p. 96 of this paper the author mentions, in a footnote, that on the trial plots where he had found the rye race of the parasite attacking lupins and rye, it also occurred on zandwikken, which is the Dutch word for *Vicia villosa* Roth. There is no mention anywhere in Mayer Gmelin's paper of voederwikke or *Vicia sativa* and the conclusion to which one is driven is that Ritzema Bos made a mistake in citing *Vicia sativa* for *Vicia villosa* as the host found by Mayer Gmelin.

Search of the relevant literature which the writer has made has failed to reveal any previous report of stem eelworm infestation on *Vicia sativa* and since, as has just been shown, the record made by Ritzema Bos in 1917 is erroneous, that presented in the paper by Staniland and Southey must be regarded as the earliest authentic record of this host/parasite association.

Experiments.

Through the good offices of Mr. F. R. Horne of the National Institute of Agricultural Botany, Cambridge, seed of both *Vicia sativa* and *V. villosa* was obtained. Pot experiments were set up on 12th April, 1950, using dried infested oat material as the inoculum. Four 5-in. pots were filled to within about 1¼ in. of the rim with John Innes compost. In two of them seeds of *V. sativa* and in two others seeds of *V. villosa* were sown. One pot of each pair was left uninoculated as control whilst the others were inoculated with chopped oat straw from the swollen bases of infested "Golden Rain" oats collected in July, 1949. On top of the inoculum a layer of soil about 1 in. deep was placed. All four pots were watered by soaking from below and were then placed in a gravel plunge.

By 15th May, 1950, i.e. 33 days from the date of sowing, seedlings of both species of vetch were showing marked symptoms of attack, which were more pronounced in the case of *V. villosa* than in *V. sativa*. Several of the seedlings in the inoculated *V. villosa* pot were very stunted and ranged in height from ¼ to 1 in., whereas in the control pot the healthy seedlings were 3½ to 4 in. high. The stunted seedlings had fewer and smaller leaflets, which were irregular in outline, twisted and somewhat thicker than normal ones. The eelworm had evidently penetrated the young tissues and had damaged them severely. Small brownish discoloured areas were found on the stipules, the shortened internodes and the deformed leaflets and numerous specimens of *Ditylenchus dipsaci* were discovered on dissecting these areas under the microscope. Similar effects were shown by the *V. sativa* seedlings, but the crippling and damage caused by the invading eelworms were not quite so marked as in the *V. villosa* seedlings. Affected and healthy seedlings of both species were lifted 37 days after sowing and drawings were made by Miss Joan Sampson at this time; see Fig. 1, which shows clearly the marked symptoms set up in an infested seedling of *V. villosa*.

Many of the *V. villosa* seedlings were killed outright but not those of *V. sativa*, and in both species several of the less severely affected

Fig. 1. Seedlings of *Vicia villosa* Roth., healthy on left, infested by the oat strain of the stem eelworm, *Ditylenchus dipsaci*, on the right. Natural size.

seedlings succeeded in producing healthy lateral shoots which grew well and ultimately masked the earlier diseased and stunted appearance shown by the plants.

Discussion.

A point of some interest and significance arising from the observations of Staniland and Southey and the present findings is that whereas Mayer Gmelin's original host record related to the rye race of the stem eelworm, the tares attacked in Gloucestershire as well as in the experiments of Staniland and Southey and in those made by the writer, infestation was due to the oat race of the stem eelworm. It may reasonably be inferred from these results that the rye race of the eelworm in Holland and the oat race in England are biologically very closely akin. Further experiments are yet needed to prove whether or not the two races are identical, but a pointer suggesting that they may prove to be one and the same biological race is to be found in the fact that rye has, this year (1950), been found to be attacked by the stem eelworm in a field in Wales where two years ago a crop of oats was a complete failure owing to stem eelworm infestation. In this connection, too, it may be mentioned that the stem eelworm attacking rye in Germany is known to attack oats also ; see Goffart (1949).

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Journal of Helminthology, Vol. XXV, *Nos.* 1/2, 1951, *pp.* 33—36.

The "Hemizonid," a Hitherto Unrecorded Structure in Members of the Tylenchoidea.

By J. Basil Goodey, B.Sc.
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During the detailed study of the finer anatomical structure of the potato tuber nematode, *Ditylenchus destructor* Thorne, 1945, the presence of an apparently hitherto unrecorded structure was revealed. It is situated on the ventral surface of the body just anterior to the excretory pore. It is quite easily seen in living nematodes and has no doubt, in the past, been confused with the excretory pore. It appears as a bright lens-like structure, more highly refractive than the neighbouring parts, especially when specimens are viewed in an approximately lateral position. The structure is really band-like, usually more or less biconvex in section, situated between the cuticle and hypodermal layer. It extends round the ventral side of the nematode, ending just short of the lateral field on either side. The deirids, when present, are to be found in the middle of the lateral fields more or less opposite the ends of this structure.

The lens-like appearance of the structure when seen in optical section suggests that a light perceptive function might be attributed to it, but without any definite evidence as to its nature this suggestion is to be avoided. The author proposes therefore, the term "Hemizonid," from $\zeta\omega\nu\eta$, a belt or girdle, describing its form without indicating in any way its function, real or imaginary.

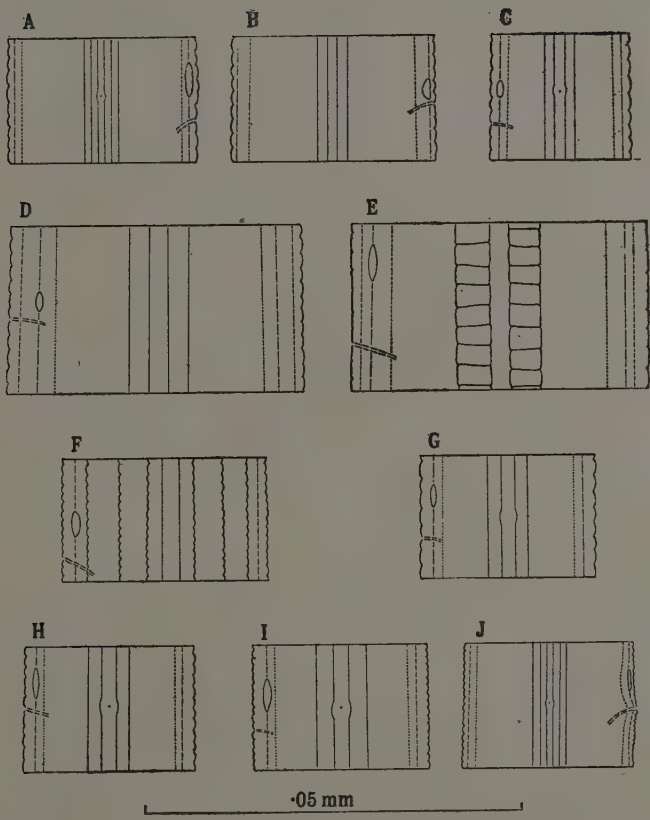
The excretory pore opens ventrally just behind the hemizonid and though the excretory duct often appears to be thinner walled in passing through the hypodermal layer before splaying out slightly as it opens through the cuticle, there does not seem to be any connection between it and the hemizonid.

Specimens from many different genera and species of plant parasitic and free-living nematodes have been examined and it appears that the possession of a hemizonid is confined to members of the super-family Tylenchoidea Chitwood and Chitwood, 1937 (see Thorne, 1949). It was not present in representatives of the Aphelenchoidea Fuchs, 1937, or in some members of the sub-class Aphasmidia which were examined. The possession of the hemizonid may prove of value as additional evidence in determining the relationships of the members of the Tylenchoidea.

Although basically the form of the hemizonid seems similar in all specimens examined, there are differences between species and also some variation even within individual species. Thus the hemizonid may be overlaid by from one to five annules of the cuticle. It varies also in position relative to the excretory pore and the level of the deirids ; it may be immediately anterior to the pore or separated from it by up to five annules. The position of the deirids varies anywhere between the level of the hemizonid and the excretory pore. Whilst the hemizonid usually appears to be biconvex in section, in *Neotylenchus consobrinus* it is apparently elongate, flat and band-like and the cuticle immediately covering it is distinctly thinner. In *Ditylenchus destructor* and *D. dipsaci* the cuticle bulges slightly over the hemizonid and the striae are extremely difficult to see, but in most cases there is no differentiation of this kind.

The following list indicates the genera and species in which the hemizonid has been observed and the accompanying figures show some of the variations in form which are met with in different nematodes.

Anguina agrostis (Steinbuch, 1799) Filipjev, 1936.
Anguina tritici (Steinbuch, 1799) Filipjev, 1936.
Ditylenchus destructor Thorne, 1945.
Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936.
Ditylenchus intermedius (de Man, 1880) Filipjev, 1936.
Ditylenchus radicicola (Greeff, 1872) Filipjev, 1936.
Hemicycliophora typica de Man, 1921.
Heterodera major (O. Schmidt, 1930) Franklin, 1940.
Heterodera marioni (Cornu, 1879) Goodey, 1932.
Heterodera rostochiensis Wollenweber, 1923.
Hoplolaimus coronatus Cobb, 1923.
Hoplolaimus uniformis Thorne, 1949.
Neotylenchus consobrinus (de Man, 1906) Filipjev, 1936.
Pratylenchus pratensis (de Man, 1880) Filipjev, 1936.
Psilenchus hilarulus de Man, 1931.
Rotylenchus bradys (Steiner and Le Hew, 1933) Filipjev, 1936.
Rotylenchus erythrinae (Zimmermann, 1904) Thorne, 1949.
Rotylenchus robustus (de Man, 1880) Filipjev, 1934.
Tetylenchus abulbosus Thorne, 1949.
Tylenchorhynchus lamelliferus (de Man, 1880) Filipjev, 1936.
Tylenchorhynchus macrurus (Goodey, 1932) Filipjev, 1936.
Tylenchus davainii Bastian, 1865.



Diagrammatic representations of the relationships of the hemizonid to the excretory pore and lateral field with incisures and deirid when present. (a) *Ditylenchus destructor*, (b) *Pratylenchus pratensis*, (c) *Heterodera marioni* ♂, (d) *Rotylenchus robustus*, (e) *Hoplolaimus uniformis*, (f) *Tylenchorhynchus lamelliferus*, (g) *Tetylenchus abulbosus*, (h) *Tylenchus davainii*, (i) *Psilenchus hilarulus*, (j) *Neotylenchus consobrinus*.

The deirid in Fig. G. has been accidentally omitted.

I am indebted to Mr. Gerald Thorne, Salt Lake City, Utah, U.S.A., for slides of *Psilenchus* and *Tetylenchus* and for various comments made after seeing a preliminary draft of this paper, also to Dr. B. Kassanis for suggesting the Greek word on which hemizonid is based.

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Journal of Helminthology, Vol. XXV, *Nos.* 1/2, 1951, *pp.* 37—48.

Investigations on the Emergence of Larvae from the Cysts of the Potato-root Eelworm, *Heterodera rostochiensis*.

4. Physical Conditions and their Influence on Larval Emergence in the Laboratory.

By D. W. Fenwick, M.Sc.

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In previous papers (Fenwick, 1949, 1950) the author dealt with the technique of inducing larval emergence from cysts of the potato-root eelworm, *Heterodera rostochiensis*, and with the variability exhibited by these organisms in hatching as well as with the form of the hatching curve. The present paper represents an attempt to investigate the effect of physical factors on larval emergence under laboratory conditions. All the experiments herein described were carried out on batches of 100 cysts, the degree of replication being governed by the statistical design of each experiment. Liberated larvae were counted by means of a dilution technique. Data for total larval emergence were submitted to logarithmic transformation prior to analysis, but all data presented in this paper, unless otherwise stated, are in the untransformed arithmetical condition. Analysis of hatching curves was by the method previously described by Fenwick (l. c.) except that, instead of expressing time as log x (x being the time in days), it was expressed as log (x + 1) ; this was found to be necessary because the class limits of the first time interval extended from 0 days to the time of the first observation, which would involve the logarithm of these class limits extending from minus infinity to some finite value, the latter representing the logarithm of the time of the first observation ; the centre point of such a class interval would therefore be indeterminate. By using log (x + 1), the new logarithmic class limits would extend from zero to some new finite value and the value of the midpoint of the class limits would become determinate.

Preliminary experiments indicated that larval emergence was influenced by a large number of factors, and this finding confirmed the observations of Calam, Raistrick and Todd (1950) who conducted an investigation into a number of such factors. Although several

other workers (Boyd 1948, Carroll and McMahon 1934, 1935 and 1937, Ellenby 1944a, 1944b, 1945 and 1946, and Triffitt 1929, 1930, 1932, 1934) have conducted experiments on the hatching of *Heterodera* cysts, there is little evidence of uniformity in the conditions under which they worked and it was decided to carry out carefully controlled experiments on the effect of varying single factors while keeping others constant.

Time of Soaking Prior to Exposure of Cysts to Diffusate.

It was generally found that a preliminary soaking of cysts in tap water increased their rate of hatching on subsequent exposure to root

Table I.

Effect of pre-soaking cysts on total hatch.

Time of Soaking	*Total Hatch*	*% Hatch*
2	1,978	75
3	2,408	78
4	2,215	75
6	2,636	79
9	2,693	76
13	2,697	80
21	2,727	76
30	2,729	82
45	3,115	81

diffusate and an experiment was carried out to investigate this effect. Batches of cysts in quintuple replication were soaked for 2, 3, 4, 6, 9, 13, 21, 30 and 45 days before immersion in root diffusate. The liberated larvae were counted 2, 4, 12, 18, 32 and 74 days after exposure to the diffusate, by which time all hatching had ceased. The total hatch expressed arithmetically and as a proportion of the total cyst contents as estimated from the total of liberated larvae and residual eggs is set out in Table I. Analysis of this data disclosed no significant difference between any of the treatments. Those cysts which had been soaked for the shorter times appeared to hatch more slowly

than did those which had been soaked for a longer period ; under the former conditions hatching occupied nearly the whole of the 74 days while under the latter the great majority of the larvae had emerged in less than 18 days. Six or more days' soaking resulted in 90% of the hatchable larvae emerging within 12 days, but soaking for 4 or fewer days resulted in at least 18 days being necessary for this degree of larval emergence. The data were submitted to probit analysis and the two parameters of the probit lines determined, viz. $\bar{x}$ the time necessary for the emergence of 50% of the hatchable larvae as well

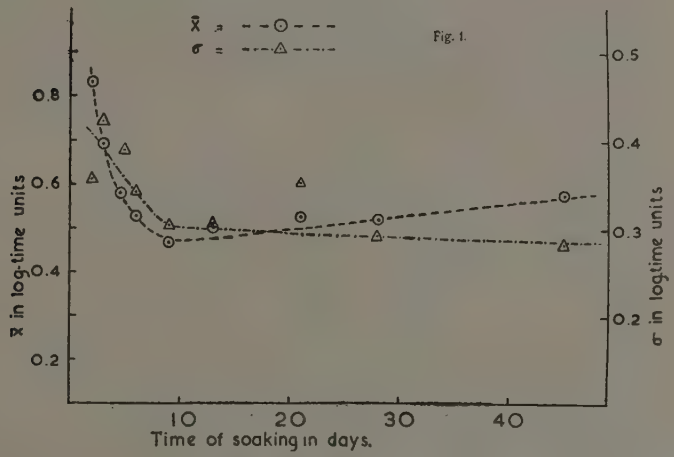

Fig. 1. The effect of pre-treatment soaking on the parameters of the hatching curve.

as σ the standard deviation of their susceptibility to root diffusate, expressed in logarithmic time units. The results of this analysis are set out graphically in Fig. 1. It will be seen that the former parameter falls rapidly as soaking time is increased from 2 to 10 days, and then shows some evidence of increasing slightly as soaking time is further increased, although it is doubtful whether this latter increase is significant. The same trend is discernible for the second parameter —a sharp fall as soaking time is increased to 10 days although, excluding the anomalously high value for 21 days' soaking, this is

followed by a further slight fall up to the maximum soaking time of 45 days. It therefore appears that the maximum hatching rate is obtained after about 10 days' soaking and that further soaking confers no advantage but may actually slightly inhibit hatching. The general conclusion to be drawn from this experiment is that soaking of cysts prior to treatment is without effect on the number of hatchable larvae, but that soaking for a period of about 10 days increases the rate of hatching to a maximum. Under these conditions, 50% of the hatchable larvae in the experimental cysts under discussion can be expected to emerge in 3–4 days. Further repetition of this experiment on other cysts using different root-diffusate samples confirmed these results although the time necessary for 50% emergence varied somewhat and in some cases rose to 8–9 days. In all cases, 10–12 days' soaking resulted in the fastest hatching.

Table II.

Effect of temperature on total hatch.

Temperature ..	15°C.	20°C.	25°C.	30°C.	Total
Gamlingay cysts ..	113	161	170	17	461
Rothamsted cysts ..	905	969	1,158	8	3,040
Total	1,018	1,130	1,328	25	3,501

The Effect of Temperature on Hatching.

Preliminary experiments having shown that hatching ceased at temperatures exceeding 30°C., four temperatures were tested, 15°C., 20°C., 25°C. and 30°C., on cysts from two sources, Gamlingay and Rothamsted. Replication was fivefold. At the highest temperature, very little hatching was observed but at the three lower temperatures there appeared to be little effect on the total hatch. The rate of hatching increased however with rise of temperature and the highest rate of hatch occurred at 25°C. The data for total hatch is set out in Table II, the letters A to D denoting successively higher temperatures from 15°C. to 30°C. Analysis of logarithmically transformed data disclosed a significant fall at 30°C. but there was no significant difference in the total hatch for the other three temperatures. Gamlingay cysts gave a lower total hatch than did Rothamsted cysts ; a significant interaction was disclosed by the analysis in that for the three

lower temperatures the difference between Gamlingay and Rothamsted cysts was maintained while at 30°C. it disappeared due probably to the fact that there was virtually no hatch at this temperature. Probit analysis of the data was carried out and the parameters $\bar{x}$ and σ estimated. The summarised results of this analysis are set out in Fig. 2.

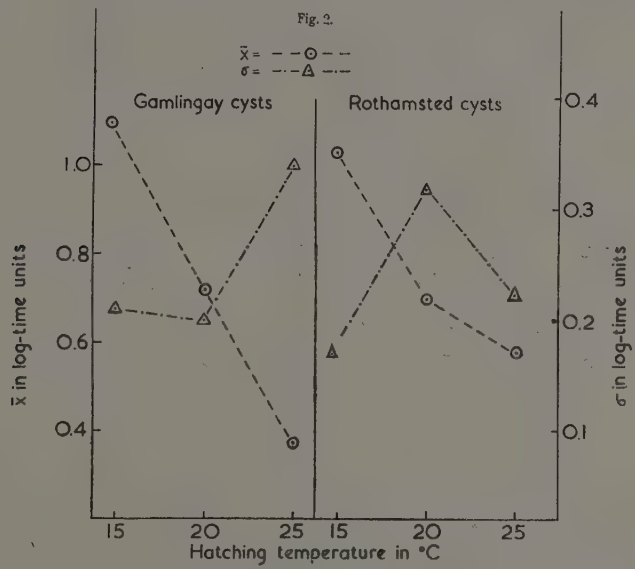

Fig. 2. The effect of different hatching temperatures on the parameters of the hatching curve.

It will be seen that the time of emergence of 50% of the hatchable larvae fell from 11.9 days for Gamlingay cysts and 10.1 days for Rothamsted cysts to 2.4 and 3.8 days respectively as the temperature was raised from 15 to 25°C. No corresponding effect is discernible in the relation of σ to temperature, the form of the graph for Gamlingay cysts and Rothamsted cysts being opposite to one another; it was therefore concluded that the variations in this parameter were probably random and not in fact connected with temperature fluctuations. The results of the temperature experiment can therefore be summarised by stating that a temperature of 30°C. inhibits hatching but that between 15°C. and 25°C. there is no effect on total larval emergence; rise of temperature between these limits involves an increase in the rate of larval emergence.

Influence of Volume of Diffusate per Cyst on Hatch.

To investigate this effect, varying numbers of cysts from 1 to 512, in multiples of two, were put to hatch in 1 cc. of diffusate in quadruplicate replication. The diffusate was changed at 2, 4 and 7 days and thereafter weekly. The data for total hatch are set out in Table III. Since the analysis of such data presents difficulties owing to the different weights applicable to these observations, it was decided that the effort involved in carrying out such an analysis was out of

Table III.

The effect of different numbers of cysts per cc. of diffusate on total hatch.

Cysts per cc.	*Total Larvae Counted*	*Dilution of Larval Suspension*	*Cysts per Counted Sample*	*Larvae per Cyst*
1	31	All larvae counted	1	31
2	346		2	173
4	252		4	63
8	225		8	28.1
16	351		16	21.9
32	90	1/25	1.28	70.0
64	70	1/25	2.56	27.3
128	207	1/25	5.12	40.5
256	544	1/25	10.24	53.2
528	1,433	1/25	20.48	70.2

proportion to the benefit gained; conclusions are therefore drawn directly from this table. Perusal of the first and last columns shows no obvious correlation between number of larvae emerging per cyst and the number of cysts per cc. of diffusate; it was therefore concluded that the density of cysts per cc. of diffusate was without effect on total larval emergence. No appreciable difference was detected in the rate of hatching; all the cysts started hatching more or less together, built up to a maximum and finished off together.

Effect of *p*H on Larval Emergence.

Great difficulty was experienced in investigating the effect of *p*H, due to difficulty in finding a satisfactory buffer which did not inhibit hatching. A variety of buffer solutions was tested, including borate-NaOH and borate-HCl, Sorenson's citrate-NaOH and Sorenson's citrate-HCl, Sorenson's phosphate, Walpole's acetate, and McIlvaine's phosphate-citrate standards. All these when diluted 1 : 10 with root-diffusate had an obvious deleterious effect on hatching and their use was abandoned. Subsequently attempts were made to adjust the *p*H of diffusate by adding N/10 sodium carbonate and N/10 hydrochloric acid solutions. Adjusted solutions obtained in this way were found to be rather unstable and tended to revert to their original *p*H value, but it was found that if they were readjusted three or four times, they became moderately stable and did not fluctuate more than 0.1–0.2 *p*H units. The addition of cysts to these solutions caused a further change in their *p*H value ; this difficulty was overcome by soaking the cysts in tap water, the *p*H of which had been adjusted. The tap water was changed daily and when soaking had been continued for 4–5 days, the cysts were treated with adjusted diffusate. During the hatching test, the diffusate was changed every two days ; *p*H values were tested electrically and readings were made for every sample of diffusate before use as well as before renewal. The maximum deviation for any culture was about 0.4 *p*H units and did not often exceed 0.2 *p*H units. The range of *p*H values tested was 3.0–8.0 ; values above 8.1 were not tested since above this value calcareous deposits were thrown down from the diffusates and hatching became irregular. The results in terms of total hatch are set out as follows :—

*p*H	3.2	3.8	4.5	5.3	6.0	6.8	8.1	pure R.D.
larvae	276	258	426	421	304	461	334	451

Analysis of logarithmically transformed data between the above-mentioned limits disclosed no significant *p*H effect. Nor was there any apparent effect on hatching rate.

The Effect of Dilution.

Early experiments suggested that moderate dilution had little effect on the hatching properties of root diffusate. Accordingly a test was set up in which a natural sample of diffusate of high activity was successively diluted with tap water in multiples of four. The dilutions tested ranged from pure diffusate to one volume of diffusate in 16,384 volumes of water. Batches of 100 cysts in quintuple replication

were subjected to the action of these dilutions. Hatching curves were plotted and the residual eggs and larvae estimated by the standard hypochlorite technique. The data thus obtained were analysed by four different methods ; the number of liberated larvae was analysed without subjection to any form of transformation as well as after logarithmic transformation ; the number of liberated larvae were expressed as a proportion of the total of liberated larvae plus residual eggs and larvae and this proportion was subjected to the ϕ transformation as well as to probit transformation. The results of these

TABLE IV.

The effect of dilution on the parameters of the hatching curve.

Dilution	$\bar{x}$	σ
pure	0.535	0.282
1 : 4	0.487	0.237
1 : 16	0.496	0.226
1 : 64	0.403	0.180
1 : 256	0.503	0.255
1 : 1,024	0.465	0.258
1 : 4,096	0.438	0.288
1 : 16,284	0.396	0.258

transformations are set out graphically in Fig. 3. It will be seen that in all cases dilution results in a decrease in hatching whatever the measure adopted. This decrease appears to be more or less linear in nature until a level is reached at which hatching is about equal to that in the water control. There appears to be some evidence of an upward convexity in the form of points obtained as a result of the logarithmic transformation, whilst in the case of the probit transformation, the distribution of the points appears to be more irregular than in the case of the arithmetic curve which latter appears to agree very well with the ϕ transformation. Whatever the form of graph used, the point corresponding to the 1 : 4 dilution appears abnormally

low and it is suggested that this apparent deviation from linearity represents an anomalous value. In view of the fact that agreement with linearity is apparently equally good for an arithmetical and a

Fig. 3.

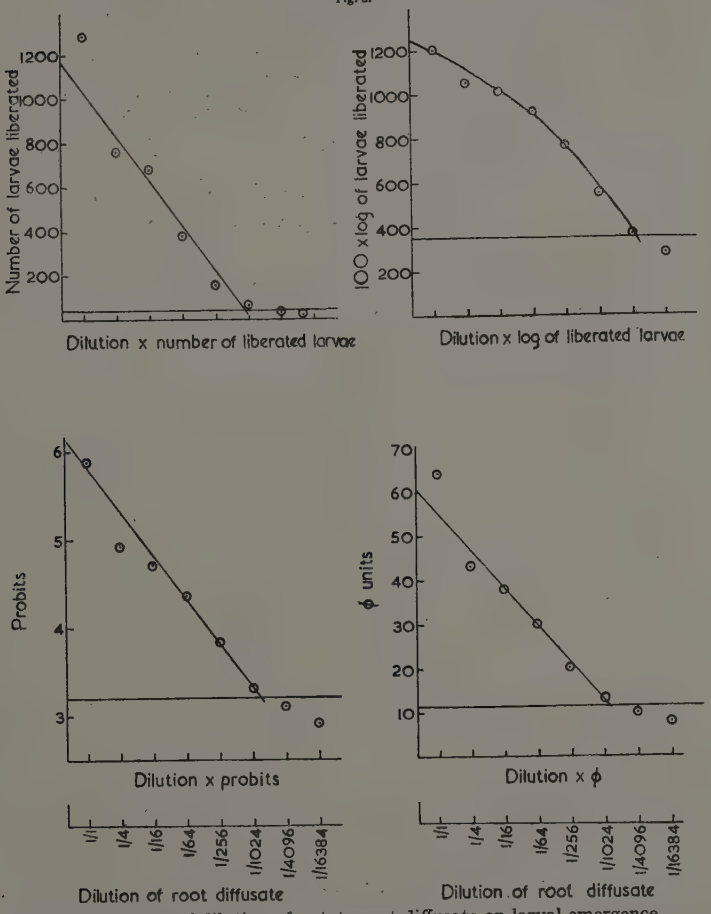

Fig. 3. The effect of dilution of potato-root diffusate on larval emergence.

ϕ graph, it is considered that the former, being simpler, is preferable to the latter. A further experiment using a different sample of diffusate on cysts from another source confirmed this view. It thus appears

reasonable to suggest that the total number of larvae capable of liberation by the action of root diffusate is proportional to the concentration of the latter when that concentration is measured geometrically.

The hatching curves for each dilution were analysed to estimate the parameters $\bar{x}$ and σ. The results of this analysis are set out in Table IV and it will be seen that there is little if any correlation between either parameter and the degree of dilution. There is an apparent tendency for both parameters to fall to a minimum value at a dilution of 1 : 64 but it is doubtful whether this decrease is significant. It is also difficult to imagine what importance could be attached to it ; in any case, the low value for $\bar{x}$ for a dilution of 1 : 64 is still higher than the corresponding value for 1 : 16,384. On the available data, it is unwise to draw any definite conclusions regarding the relationships between these parameters and the degree of dilution, especially as other dilution experiments have since presented a by no means consistent picture.

Effect of Light on Hatching.

Hatching of cysts in soil normally occurs in the dark and it is suggested by Lownsberry (1950) that light inhibits hatching. To investigate this point cysts were exposed to the action of diffusate at equal temperatures in the dark, in diffused daylight and in bright sunlight. Although active hatching occurred under the two former conditions, there being no significant difference between them, there was no emergence in bright sunlight. Subsequent transfer of such cysts to the dark did not result in hatching taking place.

Summary and Conclusions.

The foregoing results throw light on the conditions under which hatching tests can be carried out. In the first place, pre-soaking cysts in water for a period of 10 days increases the rate of larval emergence but has no effect on the total number of hatchable larvae. Increase in temperature has a similar effect up to 25°C., but at 30°C. hatching is virtually inhibited. Volume of diffusate per cyst is without effect either on the number of hatchable larvae or on their rate of hatching. Neither was any effect observed from alterations in pH between 3.2 and 8.1. The effect of dilution is more or less linear when dilution is plotted geometrically against either the number of hatchable larvae or the latter expressed as a proportion of the original cyst contents and subjected to angular transformation. For the effect to be appreciable, dilution must be of a high order. Dilution to a half or a quarter

of the original concentration has little appreciable effect. The effect of dilution on the rate of hatching is not obvious and the variations in it resulting from dilution are probably random. Direct sunlight inhibits hatching, diffused daylight is without effect as compared with darkness ; cysts exposed to the action of sunlight and diffusate do not hatch when later removed to the dark. It appears reasonable to assume that this may be due to some lethal effect.

In view of these results, it is reasonable to suggest suitable conditions for the conduct of hatching tests.

1. Cysts should be soaked for a period of 10–12 days before exposure to diffusate.

2. Tests are best conducted at a temperature of 25°C.

3. Volume of diffusate per cyst being unimportant, 1 cc. per batch of 100 cysts is quite satisfactory.

4. In view of the variability exhibited in hatching rates under different conditions, it is important that tests be continued at least long enough for some estimate of the parameters of the hatching curve to be obtained.

5. Care should be exercised when setting up water controls to ensure that all vessels and equipment used are free from contamination with diffusate in view of the activity of the latter at high dilutions.

6. Hatching tests should be conducted in the dark since bright sunlight may be lethal to the cyst contents.

Acknowledgments.

The author is indebted to Miss Elizabeth Reid for help with the numerous counts involved in this work. She also undertook a large proportion of the computations on which the statistical analyses are based.

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Investigations on the Emergence of Larvae from the Cysts of the Potato-root Eelworm, *Heterodera rostochiensis*.

5. A Shortened Method for the Conduct of Hatching Tests.

By D. W. Fenwick, M.Sc.

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Immersion of cysts of *Heterodera rostochiensis* in potato-root diffusate results in larval emergence which may occupy up to two or three months. In a previous paper (Fenwick, 1950) the author dealt with the form of the hatching curve, and showed that any given curve was controlled by three parameters ; the number of larvae finally emerging, the mean reaction time of the hatchable larvae and the standard deviation of that mean reaction time expressed in log-time units. Since it was also shown that each of these parameters was subject to considerable variation, it is obvious that maximum information and accuracy can only be obtained by continuing a hatching test until all larval emergence has ceased and plotting cumulative hatches at selected intervals of time against log-time ; in this way detailed information may be obtained regarding the parameters of the hatching curve. The futility of allowing a given test to continue for any arbitrary pre-determined time is obvious. In many cases, however, the time and labour expended in carrying on a test to completion renders such a procedure impracticable, and the time saved by resorting to an abbreviated form of test is of greater importance than a moderate resultant loss of accuracy. It is essential, however, if such a shortened method is used that the principles underlying it should be sound and that estimates obtained by means of it should be shown to bear some reasonable agreement with the values obtained by the longer test. The present paper represents an attempt to state some of the principles which apply to hatching tests with a view to evolving a suitable form of abbreviated test.

Principles Underlying Abbreviated Hatching Tests.

Suppose a batch of cysts be subjected to the action of a hatching factor such as potato-root diffusate and the total hatch at specified intervals of time be plotted against time—the latter expressed arithmetically. The form of the curve will approximate to that shown in Fig. 1—that is more or less sigmoid in character except that it becomes progressively flatter as the time scale is lengthened. It is not symmetrical about its point of inflection, the time interval necessary to cause a given increase beyond the point of inflection being far greater than that before it. If a cumulative hatch be plotted against a logarithmic function of time usually log $(x + 1)$, x being the time interval in days, then a symmetrical sigmoid curve results, as in Fig. 2. The two halves of this curve about the point of inflection are (within the limits of error) identical but opposed. The slope of the line at the point of inflection for any given scale must be dependent to a degree on its height, i.e. on the total number of larvae liberated, but if instead of plotting the numerical value of the hatch, the number of larvae emerging at the end of each interval of time be plotted as a proportion of the total hatch, then the curve will still be truly sigmoid, but discrepancies in slope due to differences in total hatch (other factors being equal) will be eliminated, and unless there are present differences in rate of hatching then two curves from data varying widely in the total numbers of larvae hatching will approximate closely to one another. If the larvae hatching at any given time be expressed as a proportion of the total hatch and this proportion be converted to probits and plotted against log-time then a straight line will result. This line will have two parameters, the mean corresponding to a probit value of 5 and a standard deviation which will be the increase in log-time corresponding to an increase of one in the probit value—in other words position and slope. The two parameters correspond in point of fact to the value of log-time at which 50% of the larvae emerge, and the standard deviation in terms of log-time of the reaction times of individual larvae to the action of the hatching factor. The three parameters previously described can thus be estimated.

Suppose now, that a batch of cysts be exposed to the action of potato-root diffusate and daily records of the number of larvae emerging be kept. If cumulative hatches are plotted against log-time, then the initial stages of the sigmoid can be traced. If the graphing is continued as far as the point of inflection and a little

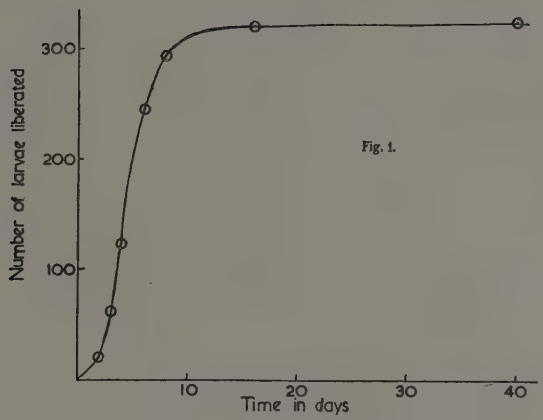

Fig. 1. Effect of plotting cumulative hatch x time.

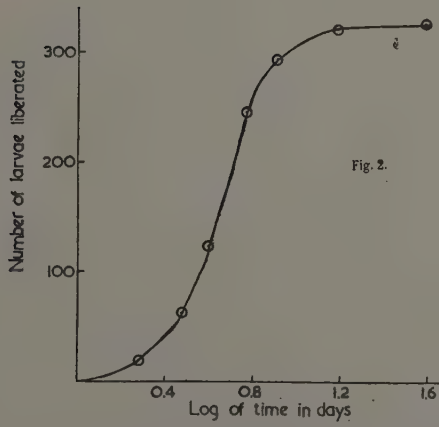

Fig. 2. Effect of plotting cumulative hatch x log-time.

beyond, then the point of inflection can be determined with a reasonable degree of accuracy.

The number of larvae liberated at this point is then an estimate of half the number which will ultimately emerge ; the log-time value corresponding to this hatch is an estimate of the mean hatching time in log-time units.

Using the computed value for the total hatch (i.e. double the number of larvae liberated at the point of inflection) it is possible to express the observed hatch at the end of each time interval as a proportion of the total. This converted to probits gives a linear probit curve for the hatch in each subtreatment. The three parameters for the hatching curve can thus be obtained. While it is quite usual for the number of larvae liberated at the point of inflection to vary widely according to the hatching factor used, and also for the log-time value for the point of inflection to vary, it is less usual for there to be great differences in slope between probit lines and, therefore, while it is of the utmost importance to pay attention to the number of larvae liberated at the point of inflection, as well as to the position of the point of inflection on the log-time scale, it may be considered unnecessary to pay a great deal of attention to the slope of the probit curve.

Procedure.

It is now possible to set out recommendations for the conduct of abbreviated hatching tests. Detailed observations should be made of the number of larvae emerging at the end of 2, 3, 4, 6, 9, 12 and 18, etc., days and the emerged larvae plotted against log $(x + 1)$, x being time in days corresponding to each larval emergence. The observations need only be continued until the point of inflection is passed for each subtreatment. The number of larvae liberated at each point of inflection together with the corresponding log-time value are recorded. The following method of determining the point of inflection is suggested. In Fig. 3, AB represents the best fitting portion of a sigmoid drawn by eye to fit the given points. The line DC is drawn through the point of inflection to correspond to the slope of the line at this point. The lines EF and GH are drawn parallel to CD and an equal distance from it to cut the sigmoid at I and J. The rectangle IJKL is then completed and the point O represented by the intersection of the diagonals of this rectangle then gives the point of inflection in terms of larvae liberated and log-time.

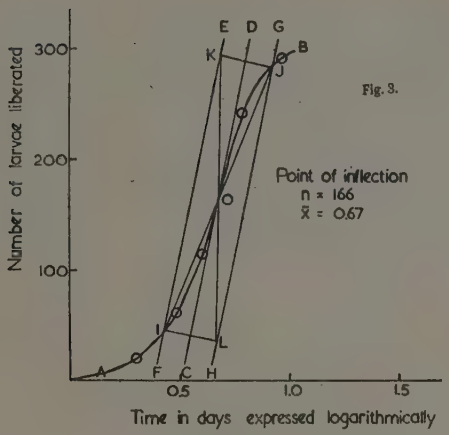

Fig. 3. Cumulative hatch for 0–8 days against log-time to estimate the point of inflection.

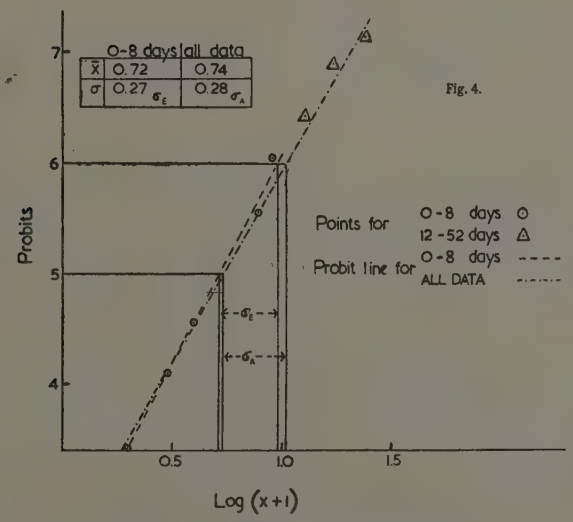

Fig. 4. Probit line for data in Table I.

It will be seen that if a series of tests is set up and the points of inflection of each are at different points on the log-time scale, then the critical counts will not be made on all the subtreatments at the same time. In point of fact there is no justification whatever for choosing any arbitrary time to apply to all members of a series of tests, since if two subtreatments vary in their hatching rates, then any attempt to apply a fixed time to all is, of necessity, bound to introduce serious errors since such counts would not represent a fixed proportion of the final total hatch.

TABLE I.

Data for larval emergence at different specified times.

Times of Observation (x)	*Log (x + 1)*	*Hatch in each Interval*	*Cumulative Hatch*	*% of Total*	*Probit*
1	.301	19	19	5.6	3.41
2	.477	43	62	18.1	4.09
3	.602	54	116	33.9	4.58
5	.778	127	243	71.2	5.56
8	.954	49	292	85.3	6.05
12	1.12	24	316	92.2	6.42
16	1.23	16	332	97.1	6.90
24	1.39	8	340	99.5	7.17
36	1.56	2	342	100	—
54	1.74	0	342	100	—

The use of this method results in a considerable saving in time over the longer method of conducting a hatching test to completion since although the log-time value for 50% emergence is half the corresponding value for 100% emergence, it is far less than that in terms of days; the former being, in point of fact, the square root of the latter. Usually a saving of at least 70% is made by use of this method.

To illustrate the method, the following example is given. Table I gives data for larval emergence in each of ten specified intervals of time. Consider only the data in the first five intervals, that is up to 8 days. In column 1 is entered the arithmetical value in days of the times of observation (x). In column 2 is the value of log (x + 1). The number of larvae emerging in each time interval is recorded in column 3 and is expressed cumulatively in column 4. A graph is then drawn (Fig. 3) in which column 2 is plotted horizontally against column 4 vertically and the curve AB obtained. The point of inflection is obtained by the method already mentioned and corresponds to an estimated 50% emergence of 166 larvae at a log-time value of 0.71. A total degree of emergence of 332 larvae can therefore be expected from this test. Should it be desired, a probit line can now

TABLE II.

Total larval emergence as estimated from the point of inflection compared with the total emergence when hatching is completed.

Estimated ..	2,700	2,840	2,040	2,560	2,200	3,040	1,960	2,940	2,390
Actual ..	2,693	2,693	2,215	2,636	1,976	3,115	2,408	2,727	2,727
Error	7	147	175	76	222	75	438	213	337
% error ..	0.26	5.46	8.57	2.89	11.2	2.41	22.3	7.81	12.3

be plotted from this data. Using the estimated value of 322 hatchable larvae, the cumulants of column 4 are entered in column 5 as a proportion of this value ; these proportions are then entered as probits in column 6. A probit line can then be drawn directly from columns 2 and 6 to give Fig. 4. The three parameters are thus capable of direct estimation. As will be seen from the last line of the table, total emergence was 342 larvae compared with the estimated value of 332—an error of approximately 3%. The mean hatching time computed from the complete data was 0.71 as compared with an estimated value of 0.74.

This method has been tested out several times and a satisfactory degree of accuracy has consistently been attained. To illustrate the accuracy attained by means of it, data are presented in Table II in which results obtained by means of the abbreviated data are set out

together with results obtained when the tests were carried out to completion. It will be seen that rarely does the error exceed 10%, an accuracy of 5–10% being much more usual.

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Further Studies on the Identification of *Heterodera* Species by Larval Length. Estimation of the Length Parameters for Eight Species and Varieties.

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In their preliminary paper (Fenwick and Franklin, 1942) on the identification of the cyst-forming species of the nematode *Heterodera* by means of the length of the larvae, the present authors studied methods of preparing the larvae for measurement and of making the measurements, and considered the form of sample to be taken from a population of *Heterodera* larvae which would give the most reliable estimate of the mean length of the whole population. Those studies were made on the larvae of *H. rostochiensis* only, but the ultimate purpose was to obtain strictly comparable estimates of the mean lengths of the larvae of all the known species of *Heterodera* in order to find out whether larvae of the different species could be identified by their length.

As was pointed out in the earlier paper (pp. 1–2), only the two species with rounded cysts, *H. rostochiensis* and *H. punctata*, can be identified with certainty from the cyst. The other species, in which the cysts are lemon-shaped, and of which at least 8 are now recognised by various writers, cannot all be identified merely by the external characters of the cyst, although, as pointed out by Goffart (1930, 1934) certain species do show characteristic variations in colour, size and shape of the cysts. These characters, however, are very variable, and individuals may be found intermediate in form between two species, or actually more characteristic of some other species than their own. In these circumstances all that can be done is to compare the contents of the cysts, that is, the eggs and their contained larvae.

In shape and size the eggs vary only slightly from species to species. The greatest difference is shown by *H. göttingiana*, in which the egg is distinctly more barrel-shaped than in any of the others, and, moreover, has a mean breadth of 54μ, while none of the others of which the measurements are known exceeds 50μ in breadth. As far as is known to the authors, however, no other species shows any character in the egg which distinguishes it from all the others.

The only possibility of identifying a given cyst thus seems to be by examination of the contained larvae. Morphologically the larvae of the various species of *Heterodera* are very much alike, but there are two characters which might prove useful, or even diagnostic in identifying them. These are the form of the buccal stylet and lips, and the length of the larva. If specific differences could be found in the mouth parts, this would make measurements of the length of the larvae unnecessary. Larvae from the following eleven populations were therefore fixed and mounted in acetic-formalin fixative or water and closely examined and compared under high magnification :—*H. rostochiensis*, *H. punctata*, *H. major*, *H. göttingiana*, *H. cruciferae*, *H. humuli*, *H. schachtii*, *H. schachtii* var. *trifolii* (from both clover and dock roots) *H.* ? sp. from carnation and *H.* ? sp. from Myosotis. At the same time the lateral line was searched for and observed where possible. It was found that there was some variation in the shape and size of the knobs at the base of the stylet, but no differences could be seen in the lateral line in those species in which it was observed. Three species seemed to differ from the majority in the form of the stylet, namely *H. rostochiensis*, *H. humuli* and *H. göttingiana*. Although in the other 8 populations it was at first thought that certain features were characteristic of certain populations, when more larvae were examined and the stylet was observed from various angles, these features could be observed at different times in larvae from most of the populations. The difference distinguishing *H. rostochiensis*, *H. humuli* and *H. göttingiana* lies in the knobs at the base of the stylet. In these species, particularly in the first two, the knobs are distinctly smaller than in the other species examined. Further, *H. rostochiensis* may be identified by the downwardly sloping " shoulder " of the knobs when seen from the side. In *H. humuli* the " shoulder," seen laterally, is slightly cupped or concave in outline. In *H. göttingiana* the shape is similar, but the whole stylet is rather stouter, including the knobs, and approaches in form and size the stylet in the other 8 forms examined.

A larva could probably, therefore, only be identified by its stylet if it were *H. rostochiensis* (which can also be identified from the cyst, if present), *H. humuli*, or *H. göttingiana*, the last being distinguished chiefly by size. Since this character is more difficult to measure accurately than the length of the whole larva, and anyhow would probably be of use in identifying only 2 or 3 of the more easily identified species of *Heterodera*, we return to the length of the larva as the only character by which it might prove possible to identify larvae of the majority of species.

In their former paper the authors worked out details of a technique by means of which, they believe, consistent and accurate estimates of the length of larvae may be made. They used this technique to measure 3,500 larvae of *H. rostochiensis*, and from the results, calculated parameters for the length of the larvae of this species. The next step to be taken was the use of this method to obtain similar parameters for larvae of as many other species of *Heterodera* as possible. A comparison of such parameters could then be made to determine whether they are of use for the identification of larvae. The present paper gives the results of the work which has been done along these lines.

Method of Measuring and Source of *Heterodera* Populations used.

Measurements of *Heterodera* larvae from as many sources as possible were made. Most of the cysts from which larvae were obtained were removed individually from the roots of their host plants, so that there could be no doubt as to their origin. The exceptions concern certain wheat plants on which insufficient cysts could be found; these plants had been grown separately in small plant pots, and had died down some time before the cysts were looked for. To complete the number of cysts required the soil from each pot was shaken up with water and the cysts which floated were collected. From amongst these a sufficient number which appeared to have been newly formed were picked out and larvae from them were measured, it being considered highly probable that they had become detached from the dead roots of the plant which had been growing in the soil. Before the larvae were obtained from any cyst which had been stored dry it was well soaked in water, and cysts freshly removed from plant roots were also kept in water until required.

As a great deal of experience had been gained in the preparation and measuring of larvae the method could safely be simplified, and may be briefly described as follows:—a single cyst, either previously soaked or freshly removed from the host root, was placed in a small drop of water on a slide. With the small glass "pestle" or "squasher" described in the previous paper, the cyst was gently crushed open and a number of the eggs were lightly pressed to release the larvae. The slide was then held over the by-pass flame of the Bunsen burner until the water had been heated sufficiently to cause the larvae to become relaxed. It is at this point that experience and great care are essential if this quick, but rather rough method of straightening the larvae is to be used, as overheating will cause them to shrink. With experience

it is possible to judge by their appearance whether the larvae have been overheated or not. If there was any doubt on this point the slide was either discarded or a drop of 1/10,000 neutral red solution was added to the water and the slide was set aside for 10 minutes. As described in the previous paper (pp. 27-29) both overheated and mechanically damaged larvae are stained by neutral red. Measurements were made only of larvae which did not become stained. The measurements were made under a monocular microscope with a micrometer eyepiece about 30 minutes after the larvae had been " fixed " by heat in the manner described above. Care was taken to ensure that the drop of water in which the larvae were measured had a fairly flat surface as it had been proved previously (pp. 8–11) that considerable magnification may occur in convex drops.

Using this method, larvae were measured from eight populations of *Heterodera* grown on the hosts given in the following list :—

I. *H. schachtii*

1. Mangolds grown on an infested plot at Winches Farm, St. Albans, during 1943.
2. Cabbages grown in plant pots of Scottish soil out-of-doors during 1942 ; cysts stored and measurements made in 1943.
3. Cauliflower plants grown in the same way, in soil from the same source.
4. Savoy cabbage, ditto.
5. Sprouting broccoli, ditto.
6. Brussels sprouts, ditto.
7. Rape, ditto.

II. *H. schachtii* var. *trifolii*

1. *Trifolium pratense*, Broad Red clover, grown in pots in cool greenhouse, 1943.
2. *T. pratense*, Montgomeryshire Red clover, single-cut, late-flowering, as above.
3. *T. pratense*, English single-cut, late-flowering, as above.
4. *T. repens*, Kentish wild white, as above.
5. *T. repens*, New Zealand wild white, as above.
6. *T. repens*, white Dutch clover, as above, 1943–1944.
7. *Rumex* sp. grown as above.

III. *H. schachtii* var. *galeopsidis*

1. *Galeopsis*: 7 plants *G. speciosa* and 2 *G. tetrahit*, grown in pots in cool greenhouse in soil sent from Cambridge by F. G. W. Jones; grown in 1943, stored dry and measured in 1944.
2. *Stellaria media*, as above.

IV. *H. göttingiana*

1. Pea var. " Onward " grown in infested plot at Winches Farm, St. Albans in 1943, cysts taken in June.
2. Pea " Onward " grown in same plot July-September, 1944.
3. Pea " Pilot," ditto.
4. Broad bean grown in same plot 1944, dug November when eggs in many cysts were still unembryonated.
5. Pea " Quite Content " grown as above.
6. Pea " Ryder's Exhibition," ditto.
7. Pea " Yorkshire Hero," ditto.
8. Pea " Gradus," ditto.

V. *H. major*

1. Oat var. " Marvellous " grown in pots in cool greenhouse, in 1943, in soil infested with cysts sent by C. R. Millikan from Victoria, Australia, in 1941; cereals had been grown in same pots of soil in 1942.
2. Wheat " Little Joss " in same soil as above in 1944. Insufficient cysts found on roots (Nov.) so soil from each pot separately shaken with water and sufficient new-looking cysts taken from the floating material.
   Rest returned to soil for no. 7 below.
3. Oat var. " Marvellous " in pots of soil from infested plot at Winches Farm, grown in cool greenhouse, 1943.
4. Oat from Cheshire, sent by Dr. M. Cohen, 1944.
5. Oat from Shropshire, 1942; cysts stored dry and measured after soaking in 1943.
6. Rye var. Large-grained Winter Rye, in pots of soil from infested plot at Winches Farm, grown in cool greenhouse in 1945.
7. Rye, same variety as above, grown in same soil as no. 2 above, in 1945.

VI. *H. cruciferae*

1. Cabbage in pots of infested soil in cool greenhouse, July-December, 1943, larvae measured December, 1943.
2. Radish in soil from same source, same conditions, 1943-1944.
3. Cabbage plants from Yorkshire, sent by H. W. Thompson, October, 1944, many of the eggs not embryonated, so roots buried in moist sand till February, 1945, when sufficient cysts with embryonated eggs were found, and measurements made.

VII. *H. humuli*

1. Hop roots from East Malling, April, 1943.

VIII. Carrot *Heterodera*

1. Carrots grown in small pot of soil infested with this species, provided by F. G. W. Jones.

In Table I will be found the numbers of cysts, and of plants from which they were obtained, from which measurements of larvae of the above 36 populations of *Heterodera* were made. In all, 10 larvae from each of the 2,312 cysts were measured, the cysts being of 8 different species and varieties of *Heterodera* and coming from 267 plants in 36 different lots.

This type of work could be extended almost indefinitely to include measurements of larvae from many different localities and host plants. But such data would quickly become unwieldy, and the authors felt that sufficient material had been accumulated for an analysis to be made which would give reliable estimates of parameters of the length of larvae of the available populations.

This data is analysed in the following section.

Analysis of Data and Results Obtained.

The analysis of data appertaining to the lengths of larvae in a specified sample of *H. rostochiensis* was dealt with by the authors in their previous paper (pp. 37–43). The sample was derived from seven varieties of potato plants grown in infested ground. Five plants were taken from each variety and ten cysts were removed from each plant and opened in order that ten larvae could be measured. The analysis of this data was simple since it took on the form of a " Chinese Box " in which smaller " parcels " or cysts were grouped to form larger " parcels " or plants which were in turn grouped to form varieties and so on. Moreover all " parcels " of the same order were of the same size ; there were always 10 larvae per cyst, 10 cysts per plant and 5 plants per variety.

In the present case, however, a complication was introduced because the authors were not able to choose the exact form of their samples since they could not forecast what material would be available for examination. They therefore examined all available material, a limit being set at 10 cysts per plant, and the number of larvae per cyst being kept constant at 10. The resulting data, therefore, lost the symmetry characteristic of that described hitherto, there being a variable number of cysts per plant, and plants per host variety.

It was difficult to decide on the best method of estimating the mean length of larvae for any given species of parasite under these changed conditions. In the symmetrical arrangement previously described all means of a given order had a constant weight, e.g. plant means were always based on 100 larvae and host variety means on 500 larvae ; consequently the computation of the mean merely involved a summation of all larval lengths within the sample and a subsequent division by the total number of larvae measured. The figure thus obtained would constitute the best estimate possible of the mean length of a general population of larvae, since all host varieties examined and all plants within varieties had been given equal weights. However, in the present case the only means with a constant weight were cyst means, all higher order means varied in weight according to the number of larvae from which they were derived. There appeared to the authors to be two courses open : one was to sum all values of larval lengths in a given sample and divide by the total number of larvae in that sample ; the other, to give to each plant mean an arbitrary weight of unity, then sum these means for each host variety and compute a separate variety mean, giving these means in turn a weight of unity and computing a species mean.

Long, involved and somewhat academic arguments can be advanced for and against either of these methods into which the authors do not feel inclined to be drawn. They would merely point out that the two alternatives could be justified on the basis of two alternative assumptions. Consider first the former procedure of dividing the sum of all values by the number of larvae in the sample—i.e. giving to each mean its own actual weight ; the result of this would be that the final mean obtained would be biased in favour of host varieties or plants from which most larvae were examined. To apply this mean to a generalised population would involve the assumption that the form of the generalised population followed the form of the sample—a completely unwarranted assumption. On the other hand if the second course were adopted it could only be based on the assumption that (a) the form of

the sample examined bore no relation to the form of a generalised population and (b) in a generalised population larvae from all host species were equally abundant. Whilst being more than willing to agree to the first part, the authors can find no grounds whatever to justify the second part of this assumption. In the absence of any information regarding the form of a generalised population of eelworm larvae the authors would hesitate to express any opinion on the validity of either procedure. In point of fact the former was adopted, the lengths of all larvae measured within a given species of *Heterodera* were summed and the grand total thus obtained was divided by the total number of larvae measured to arrive at the grand mean. The outstanding argument in favour of this procedure is its inherent simplicity.

A second complication arose in the computation of variances. In the authors' previous example an analysis of variance was made and the symmetry of the data rendered it easy to analyse any particular item in the table into its component parts, and thus remove from any high order variance those portions which were contributed by lower order variances, and so obtain estimates of the absolute variance applicable to each level of variability. The asymmetrical nature of the present data rendered it impossible to do this by any simple method. For the method of analysis of such data the authors are indebted to Mr. G. V. Dyke of the Statistical Department, Rothamsted Experimental Station, who has been good enough to publish it as an appendix to this paper. It will be seen that essentially the method consists of formulating a series of simultaneous equations, the coefficients of which are derived from the numbers of larvae involved at different stages, the unknowns being estimates of the absolute variances attributable to different levels of variability such as " larval means within cysts " " cyst means within plants," etc.

The derivation from these parameters of estimates applicable to known and specified populations is discussed in the authors' preceding paper and need not be dealt with here. Suffice it to say that two types of parameters were calculated ; (a) the standard deviation of means derived from different sized samples of larvae all independent of one another (which reduces to the form $\sqrt{\frac{\sigma^2S+\sigma^2P+\sigma^2C+\sigma^2L}{n}}$ where n is the number of larvae in the sample and σ^2S, σ^2P, σ^2C and σ^2L are estimates of absolute variances attributable to host strains, plants within host strains, cysts within plants and larvae within cysts) and (b) the standard deviation of means derived from different sized samples of larvae when

all the larvae in a sample are derived from a single cyst (which parameter reduces to the form $\sqrt{\sigma^2S+\sigma^2P+^2C+\sigma^2L/n}$ the symbols having the same significance as before).

In Table I it will be seen that in all, eight species and varieties of *Heterodera* forming lemon-shaped cysts have been examined and for each of them varying numbers of different hosts have been considered.

To illustrate the derivation of parameters a specimen analysis of the data relating to *H. göttingiana* may be considered. An analysis of variance of the raw asymmetrical data is given in Table 2, and it will be seen that the components of variance due to " larvae within cysts," " cysts within plants," etc., are all significant, each being compared by the F test with the component of the preceding line of Table 2. Using the technique described and illustrated in the appendix the following parameters are obtained :—

$$\sigma^2L = 2.827$$
$$\sigma^2C = 2.178$$
$$\sigma^2P = 0.244$$
$$\sigma^2V = 6.835$$

The figure 6.835 represents the variance of variety means when there is no " within variety " variance. If only one plant per variety were examined and there were no " within plant " variance then varietal variance would be $\sigma^2V + \sigma^2P$, and if only one cyst per plant were examined and there were no " within cyst " variance we would get $\sigma^2V + \sigma^2P + \sigma^2C = 9.257$. If only n larvae per cyst be examined then the variance would be $9.257 + \sigma^2L/n$. Hence a table (Table 3) can be drawn up giving the " between cyst " variance for different values of n, the number of larvae per cyst. It will be realised that all data up to this stage are given in arbitrary units corresponding to divisions on the micrometer eyepiece of the microscope used. One division of this micrometer eyepiece corresponds to 9.2638μ and the last column of the table gives the value of the standard deviation in μ. Further study of this table discloses an interesting fact ; the more larvae per cyst are examined the closer does the standard deviation of cyst means approach a minimum limiting value—in other words the increase in accuracy of a determination falls as the number of larvae per cyst examined increases. Accordingly, recourse must be had to some method of increasing the accuracy of the determination other than that of increasing the number of larvae measured from each cyst. There would appear to the authors to be two alternatives—either to examine a larger number of cysts or alternatively to examine a larger number of

TABLE I

Parasite	Host	No. of Plants	Number of cysts per plant												Total Cysts
			1	2	3	4	5	6	7	8	9	10	11	12	13	
I. *H. schachtii*	1. Mangold	10	10	10	10	10	10	10	10	10	10	10				100
	2. Cabbage	7	10	10	10	10	10	10	3							63
	3. Cauliflower	2	10	3												13
	4. Savoy cabbage	4	10	7	5	2										24
	5. Sprouting broccoli	1	10													10
	6. Brussels sprouts	3	4	7	5											16
	7. Rape	4	9	9	4	2										24
II. *H. schachtii*	1. Broad red clover	10	10	10	10	10	10	10	10	10	10	10				100
	2. Montgomery red	9	10	10	10	10	10	10	6	2	3					71
var.	3. English single-cut	11	10	10	10	10	10	10	10	10	8	9	6			103
	4. Kent wild white	10	10	10	10	10	10	10	10	10	10	10				100
trifolii	5. New Zealand w.w.	9	10	10	10	10	10	10	10	10	10					90
	6. White Dutch	10	10	10	10	10	10	10	10	10	10	10				100
	7. *Rumex* sp.	8	10	10	10	10	10	10	10	10						80
III. *H. schachtii* var. *galeopsidis*	1. *G. speciosa* and *G. tetrahit*	9	10	10	10	10	10	10	10	10	10					90
	2. *Stellaria media*	13	10	10	10	10	10	10	10	10	10	10	10	10	10	130
IV. *H. gottingiana*	1. Pea "Onward"	10	10	10	10	10	10	10	10	10	10	10				100
	2. Pea "Onward"	10	10	10	10	10	10	10	10	10	10	10				100
	3. Pea "Pilot"	10	10	10	10	10	10	10	10	10	10	10				100
	4. Broad bean	10	10	10	10	10	10	10	10	10	10	10				100
	5. Pea "Quite Content"	7	10	10	10	10	10	8	4							62
	6. Pea "Ryder's Exhibition"	5	10	10	10	7	2									39
	7. Pea "Yorkshire Hero"	2	10	10												20
	8. Pea "Gradus"	2	8	6												14
V. *H. major*	1. Oat "Marvellous"	11	10	10	10	10	10	10	10	10	10	10	10			110
	2. Wheat "Little Joss"	11	10	10	10	10	10	10	10	10	10	10	10			110
	3. Oat "Marvellous"	6	7	6	5	4	4	3								29
	4. Oat, Cheshire	6	10	6	6	4	3	2								31
	5. Oat, Shropshire	3	4	4	4											12
	6. Rye, large-grained winter	3	10	7	4											21
	7. Rye, large-grained winter	7	10	10	8	3	3	3	1							38
VI. *H. cruciferae*	1. Cabbage	12	10	10	10	10	10	10	10	10	10	10	10	10		120
	2. Radish	13	8	7	7	6	6	6	5	5	4	3	3	3	3	66
	3. Cabbage, Yorkshire	6	10	10	10	10	10	10								60
VII. *H. humuli*	1. Hop, Kent	7	10	10	10	10	10	6	5							61
VIII. Carrot *Heterodera*	1. Carrot	6	10	6	3	2	2	2								25
8	36	267														2,312

independent larvae from different cysts. If the former course is adopted then the standard error will be reduced in inverse proportion to the square root of the number of cysts examined. Suppose that 5 larvae per cyst be examined, then the standard deviation of cyst means will be 29.4μ and if 2, 5 and 10 cysts be examined, all independent of

TABLE II.

Analysis of Variance of H. göttingiana *data.*

Source	SS	D of F	Mean square	Variance ratio
Larvae within cysts	13,613	4815	2.827(v_1)	
Cysts within plants	11,786.07	479	24.606(v_2)	v_2/v_1 = 10 app. (sig.)
Plants within varieties	2,558.25	43	53.297(v_3)	v_3/v_2 = 2.17 (sig.)
Between varieties	31,029.09	7	4,432.72(v_4)	v_4/v_3 = 80 app. (sig.)
Total	58,986.41	5349		

TABLE III.

Showing variability of cyst means for different numbers of larvae (n) examined per cyst.

n	σ^2	σ	σ in μ
1	12.082	3.48	32.2
2	10.668	3.25	30.2
5	9.820	3.14	29.0
10	9.538	3.09	28.6
∞	9.255	3.04	28.1

one another, then the standard deviation of the grand mean will be reduced to 20.6, 13.1 and 9.3μ respectively. It will be realised that this method can only be applied to cyst samples collected in such a way that the chance is small of several cysts from a single plant being examined. Generally speaking where a bulk sample of soil made up of several smaller samples taken from several different points is examined, then in all probability this condition is satisfied.

If, on the other hand, the second alternative is considered, then the accuracy will be proportional to the square root of the number of larvae examined, the actual standard deviation of the mean being the value for n = 1 in Table 3 divided by the square root of the number of larvae examined. Here again the increase in accuracy depends on the larvae measured being independent of one another, i.e. being derived from different cysts. The method to be used to ensure this, depends to a large extent on the operator. An obvious method is to dissect a given number of individual cysts and measure one larva from each. Alternatively many workers would prefer to dissect a large

TABLE IV.

Parameters for larval lengths of nine different species and varieties of Heterodera.

Species	Mean	Standard deviation	Coefficient of variation
H. major	582μ	27.7μ	4.76
H. schachtii var. *galeopsidis*	518μ	27.8μ	5.34
H. schachtii var. *trifolii* ..	502μ	33.6μ	6.70
H. göttingiana	474μ	32.2μ	6.80
H. rostochiensis	471μ	22.8μ	4.85
H. schachtii	459μ	27.0μ	5.87
Carrot *Heterodera* ..	451μ	23.3μ	5.18
H. cruciferae	414μ	19.6μ	4.74
H. humuli	405μ	16.0μ	3.95

number of cysts, pool the larvae, and measure a given number ; if this latter procedure is adopted it is important that the number of cysts dissected be about 4–5 times the number of larvae measured.

Fig. 1 illustrates the different results obtainable according to the method of choosing the sample of larvae to be measured ; in this the standard deviation is plotted against the number of individuals examined. The latter have been plotted on a scale where any given reading is equivalent to $1/\sqrt{n}$ i.e. a reciprocal square root scale. This method has the advantage that since the standard error of the mean of a sample of independent larvae is inversely proportional to the square root of the number of larvae measured it can be represented by a straight

line, and hence the triangles of the right-hand portion of Fig. 1 are obtained. The left-hand pair of curves shows how the standard deviation of cyst means falls as the number of larvae measured per cyst is increased. The right-hand portion shows how the standard deviation of a mean falls as the number of cysts examined is increased when 1 and 10 larvae per cyst are measured. The outer of these latter curves, corresponding to one larva per cyst, shows, in point of fact, the effect of examining varying numbers of independent larvae, since no two larvae are derived from any one cyst.

Fig. 1.

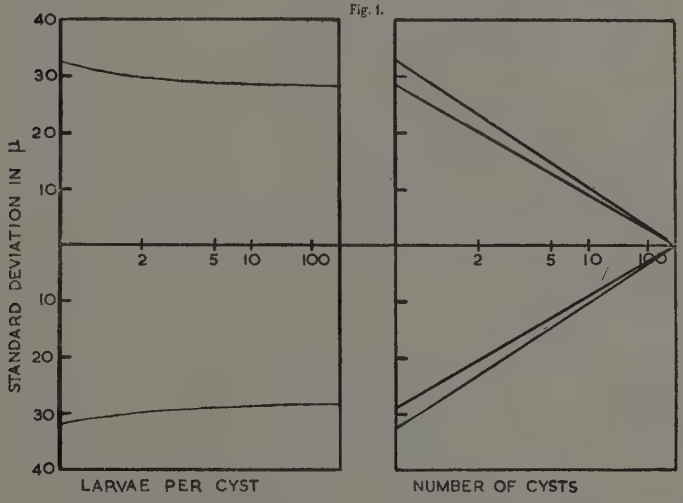

Fig. 1. Effect of measuring varying numbers of larvae per cyst and varying numbers of cysts on the standard deviation of the length of larvae of *Heterodera göttingiana*.

The computation of the mean length of larvae of *H. göttingiana* as described gave a corrected figure of 51.108 divisions corresponding to 473.45μ.

The analysis of data for the other species was accomplished in an identical manner and the results are set out in Table 4, which gives the mean length of each species and the standard deviation of a population

of independent larvae of each species. The effect of varying the number of larvae examined per cyst is shown in Fig. 2. Fig. 3 shows how the standard deviation of the mean length of several independent larvae is decreased as the number of larvae measured is increased.

It will be noted that Table 4 includes data for *Heterodera rostochiensis* as well as for the " carrot *Heterodera*." In view of the fact that *H. rostochiensis* can be distinguished by the shape of the cyst it has not been included in Figs. 2 and 3. The material for the carrot data is very scanty, comprising only 6 plants and a total of 25 cysts, which is not sufficient, in the authors' opinion, to justify its inclusion in Figs 2 and 3.

Discussion.

Consideration of Figs. 2 and 3 discloses the fact that the different species can be divided into 3 main groups as far as larval length is concerned :—*H. major* with very long larvae 550–600μ ; populations with larvae of intermediate length, comprising *H. schachtii* and its varieties as well as *H. göttingiana*, with lengths ranging from 430–550μ ; and a group with short larvae of lengths 380–430μ, comprising *H. cruciferae* and *H. humuli*. Provided that only one species of parasite is present it should be a comparatively easy matter to determine into which group it falls, since if a minimum of 4 independent larvae be measured, then there would be no overlap in larval lengths at a distance of 2 standard deviations from the mean in the case of the first and second groups, and only a very slight overlap between the second and third groups. The very fact that larvae from lemon-shaped cysts are identifiable as belonging to the first group will at once fix them as *H. major*, whilst if a population is found to belong to the shortest group, then only very rarely should it be difficult, after studying the crop-history of the soil from which it came, to decide whether or not the species present is *H. humuli* or *H. cruciferae*, since these two species have distinct host ranges.

The identification of species of intermediate length is a more difficult matter, complicated by the fact that the status of the populations tentatively regarded as varieties of *H. schachtii* is by no means certain. The fact that *H. göttingiana* occupies a position intermediate between the true *H. schachtii* and the *Trifolium* variety, renders the problem even more difficult, whilst if further observations on the carrot form confirm the preliminary indications that the mean length for this species is 450.9μ, then the very close proximity in larval length between *H. göttingiana*, *H. schachtii* and the new form, will make their separation on larval length virtually impossible. The only apparently feasible

way of identifying homogeneous populations from this group would be by increasing the number of independent larvae measured. If 50 independent larvae are examined then there should be no overlap at 2σ between *H. schachtii* var. *galeopsidis* and *H. schachtii* var. *trifolii*, nor between the latter and *H. göttingiana*, while the overlap between *H. göttingiana* and *H. schachtii* should be small. If 100 larvae are examined then this last overlap should disappear and a positive identification should be possible.

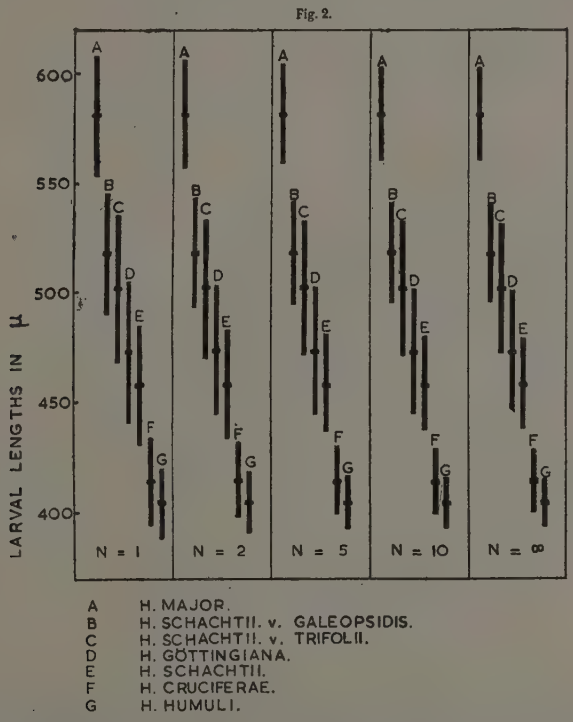

Fig. 2. Parameters for the distribution of larval lengths of *Heterodera* species when varying numbers of larvae are measured per cyst.

In the above discussion the assumption has been made that the population being investigated is homogeneous, and the question still remains how one is to determine whether or not this is so. No final answer to this question can yet be given by the authors. It is certainly

desirable that a frequency distribution be plotted and examined. The form and shape of this curve might indicate whether or not the population is obviously homogeneous or heterogeneous. If there is any doubt it will obviously be necessary to carry out a x^2 test on the normality of the data, and if there is no significant deviation from normality it is reasonable to believe that the material is homogeneous. The position

Fig. 3.

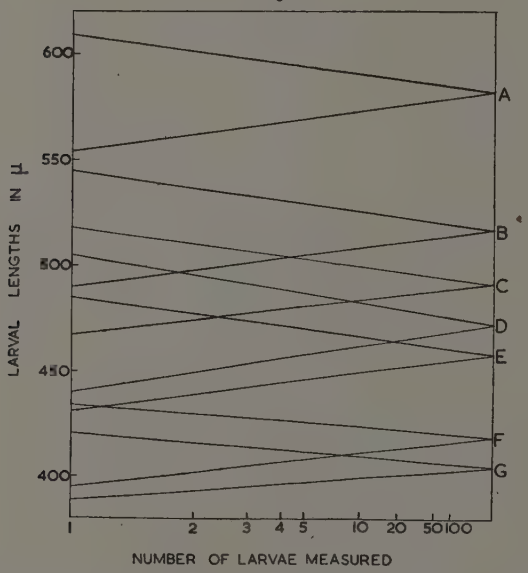

Fig. 3. Parameters for distribution of larval lengths of *Heterodera* species when varying numbers of independent larvae are measured. Lettering as in Fig. 2.

of the mean will then determine the species of parasite. When carrying out the x^2 test it will be necessary to determine the standard deviation of the sample examined and to check the agreement between this and that of the species suspected, since if there is a mixed population present it is probable that the standard deviation of the mixed population will be greater than that of a homogeneous one.

It will be obvious from the foregoing discussion that the method of measuring larvae as a means of determining the species of *Heterodera* cysts extracted from a sample of soil has its limitations. If the mean length of a suitable sample of the larvae is near the upper or the lower limits of the values which have been given above, and if the standard deviation is not abnormally high, the species can be identified with some certainty in either case as being one of two possible ones. In the case of larvae of relatively great length the species may be *H. major* (mean 582μ) or *H. punctata* (mean approximately 581μ according to Franklin 1940) : if the larvae are relatively small the species may be either *H. cruciferae* (mean 414μ) or *H. humuli* (mean 405μ). When the larvae are of intermediate length it may only be possible to identify them as being one of 3 or 4 species, unless a rather large number of measurements is made. In any case all the other characters of the nematode which can be examined, together with any facts known about the possible association over a period of 4 or 5 years of any likely host plants, must be carefully considered. For example, on the one hand, *H. major* can readily be distinguished from *H. punctata* by the character of the cyst which is dark brown and lemon-shaped in the former, but light brown and ovoid in the latter : on the other hand the probability as to whether the species in question is *H. cruciferae* or *H. humuli* can fairly safely be decided if the plant population growing in the soil is known, though it must be remembered that little is known of the host range of *H. humuli*. It must, however, be emphasized that our knowledge of the genus *Heterodera* is very probably incomplete and other species having larvae of the same order of length as those already examined may very well exist (e.g. *H. cacti* Filipjev and Schuurmans Stekhoven, 1941). Provided that the limitations of the method are borne in mind, however, valuable help towards the identification of *Heterodera* species may be gained by means of measurements of larvae carried out as described in this paper.

Summary.

Using a standard technique, larvae have been measured from the cysts of eight populations of *Heterodera* from known host plants of 36 different species and varieties. The data obtained have been analysed statistically and parameters derived for the mean lengths of larvae and their standard deviations for each population. The mean lengths of the 7 populations for which a sufficient number of larvae were measured fall into 3 groups, long, medium and short. The mean length of 4 larvae, one each from 4 independent cysts in a homogeneous

population will probably indicate to which group the population belongs. If it belongs to the group with the longest larvae and the cysts are lemon-shaped, it must be *H. major* : if it falls into the group with the smallest larvae it must almost certainly, according to our present knowledge, be either *H. cruciferae* or *H. humuli*. If the mean falls within the range 430–550μ, there is a slight chance that if it has a very low value it might be *H. cruciferae* or *H. humuli*, but in all probability it belongs to the medium group comprising *H. schachtii*, the two populations tentatively regarded as varieties of *H. schachtii*, and *H. göttingiana*, and possibly also including the carrot *Heterodera*. If the number of independent larvae measured be increased to 50 or 100 it may be possible to arrive at a decision as to the species in question, particularly if something is also known of the characters of the cysts and of the plant populations of the soil where the cysts occur. It may be, however, that other species of *Heterodera* will be found having larvae with mean length of such a size as to be inseparable from the larvae of some of the species already known.

If more than one species of *Heterodera* is present in a population the distribution of the lengths of the larvae will not fall into a normal curve, and the methods given will only serve to identify the species present under certain very special conditions.

Acknowledgments.

The authors take this opportunity of thanking those who have been good enough to send infested soil and plants bearing cysts of different species of *Heterodera* from various localities. Without this help sufficient data to make the foregoing analyses would not have been available.

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APPENDIX.

Expectations of Mean Squares in Analysis of Variance.

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n_{ijk} denotes the number of larvae in the k-th cyst of the j-th plant of the i-th variety.

J_i and K_{ij} denote respectively the number of plants of the i-th variety and the number of cysts taken from the j-th plant of the i-th variety. I denotes the number of varieties.

A dot in place of a suffix indicates summation with respect to that suffix.

TABLE I. APPENDIX.

Component of Variance	Expectation of Mean Square
Larvae within cysts	σ^2_L
Cysts within plants	$\sigma^2_C \left\{ n... - \sum_{ij}\left(\frac{1}{n_{ij.}}\sum_k n^2_{ijk}\right)\right\}/(K..-J)+\sigma^2_L$
Plants within varieties	$\sigma^2_P \left\{ n... - \sum_i\left(\frac{1}{n_{i..}}\sum_j n^2_{ij.}\right)\right\}/(J.-I)$ $+\sigma^2_C\left\{\sum_{ij}\left(\frac{1}{n_{ij.}}-\frac{1}{n_{i..}}\right)\sum_k n^2_{ijk}\right\}/(J.-I)+\sigma^2_L$
Between varieties	$\sigma^2_V\left\{n... - \left(\sum_i n^2_{i..}\right)/n...\right\}/(I-1)$ $+\sigma^2_P\left\{\sum_i\left(\frac{1}{n_{i..}}-\frac{1}{n...}\right)\sum_j n^2_{ij.}\right\}/(I-1)$ $+\sigma^2_C\left\{\sum_i\left(\frac{1}{n_{i..}}-\frac{1}{n...}\right)\sum_j\sum_k n^2_{ijk}\right\}/(I-1)+\sigma^2_L$

TABLE II. APPENDIX.

Component of Variance	Expectation of Mean Square
Larvae within cysts ..	σ^2_L
Cysts within plants ..	$10\sigma^2_C+\sigma^2_L$
Plants within varieties ..	$10\sigma^2_P\left\{K.. - \sum_i\left(\sum_j K^2_{ij}/K_{i.}\right)\right\}/\left(\sum_i J_i - I\right)+10\sigma^2_C+\sigma^2_L$
Between varieties.. ..	$10\sigma^2_V\left\{K.. - \sum_i K^2_{i.}/K..\right\}/(I-1)$ $+10\sigma^2_P\sum_i\left\{\left(\frac{1}{K_{i.}}-\frac{1}{K..}\right)\sum_j K^2_{ij}\right\}/(I-1)+10\sigma^2_C+\sigma^2_L$

The expected values of the mean squares in the various lines of the analysis of variance are given in Table I for the general case (n_{ijk} any numbers) and in Table II for the special case ($n_{ijk} = 10$) as used in this paper.

These results (obtained independently) confirm those of Ganguli (1941) subject to a small correction which becomes apparent on comparison of his formulae for the different cases considered.

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The Emergence of the Cercariae of *Fasciola hepatica* from the Snail *Limnaea truncatula*.

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At the Veterinary Laboratory, Weybridge, an investigation into the epidemiology of Fascioliasis as a disease of farm animals has included a study of the larval stages of *Fasciola hepatica* found within the snail host *Limnaea truncatula* and has involved observations on the conditions under which the most mature forms, the cercariae, leave the snail. As indicated by the observations of Agersborg (1924), different larval stages of a trematode may coexist in a single snail and in snails parasitized by *F. hepatica* it is easy to demonstrate the simultaneous presence of immature and mature rediae and of cercariae in various stages of development. It follows that all the cercariae do not mature at the same time and as demonstrated by Faust and Hoffman (1934) they leave the snail over an extended period.

Although the emergence of cercariae from any individual snail occupies a considerable period of time it is not a continuous process, groups of parasites leaving the snail at intervals. This periodic emergence of cercariae from their snail hosts and the factors causing emergence have been discussed by numerous workers, among whom may be mentioned Walton (1918), Rees (1931 and 1948), Swales (1935), Bauman, Bennett and Ingalls (1948) and Schreiber and Schubert (1949). At Weybridge we have extended such observations in an investigation of the emergence of *F. hepatica* from *L. truncatula* in the belief that knowledge of the process would be likely to assist our research into the epidemiology of Fascioliasis.

Factors Known to Influence the Development of *F. hepatica* in *L. truncatula*.

When considering the problems of emergence it is at first necessary to discriminate between factors connected with the rate of development of the parasite and those connected with the actual emergence of mature cercariae. Temperature, as shown by Ross (1930), has a most important influence on the rate of development of the parasite and

affects emergence through an acceleration of the rate at which cercariae mature. Similarly, as shown by Kendall (1949*a*), the rate of development of the parasite within its snail host is closely associated with the nutrition of the host. Lack of food is speedily reflected in a reduction of the numbers of parasites which having come to maturity are able to leave the snail. This paper considers more particularly those factors which influence the actual emergence of cercariae already mature and free in the body cavity of the snail.

Factors Influencing the Emergence of Mature Cercariae.

A. The effect of temperature.

Workers with a variety of trematodes have suggested that the emergence of cercariae is associated with changes in the temperature of the environment of the snail. Thus Cort (1922) found that a fall in temperature to 39.5°F. followed by a "cold rainy day" largely inhibited the emergence of *Cercariae elephantis*, while Archibald and Marshall (1932) stated that large numbers of cercariae could be obtained from infected *Planorbis alexandrinus* if the snails were kept at 9°C. for twenty minutes and then subjected to room temperatures. Similarly, Brackett (1940) noted that larval schistosomes could be induced to emerge by raising the temperature of the environment from 18°C. to 30°C. Our observations on *F. hepatica* have suggested that there is a minimum temperature below which cercariae will not emerge but that above this threshold indiscriminate emergence occurs over a wide temperature range.

Minimum temperature at which cercariae emerge.

Our (unpublished) observations have confirmed that below 10°C. the development of all intra-molluscan stages of *F. hepatica* is inhibited. Further observations have suggested that the emergence of fully mature cercariae is inhibited at low temperatures. No emergence was observed from snails which were kept for 16 hours at a temperature of 7°C. although, within an hour, large numbers of cercariae left snails of the same group, when the temperature rose to that of the laboratory (13.9°C.). To determine the critical temperature below which emergence would not occur snails known to contain mature cercariae were placed in water in a water bath, the temperature of which could be controlled by the addition of melting ice. Emergence, at fixed temperatures ranging from 5.25°C. to 10.25°C., was compared with emergence from a similar group of snails maintained at room temperatures (15.5°C. to 21.0°C.). Whereas, emergence from the snails

at room temperatures commenced within two hours of the snails being placed in water, emergence from the other group of snails did not commence until the temperature reached 9.0°C. At this temperature one cercaria which emerged appeared sluggish and swam slowly for nearly an hour at the bottom of the tube. At 10°C. to 11°C. the numbers of cercariae which emerged increased, their activity appeared normal and encystment was rapid. Reducing the temperature below 9°C. appeared to inhibit emergence once more although the snails themselves remained active at temperatures as low as 1°C. It seemed that 9°C. was the critical temperature below which emergence did not occur.

Maximum temperatures at which cercariae emerge.

The effect of temperatures higher than 26°C. was not observed owing to the difficulty of keeping the infected snails alive under such conditions. There was, however, no evidence that emergence was inhibited at high temperatures. In one observation, twenty uniformly infected *L. truncatula* were divided into two equal groups. Ten of the snails were moved from room temperatures to tubes of water in an incubator which was maintained at 26°C., while ten snails of a second group were placed in an ice-cooled cabinet which was maintained at a temperature of approximately 10°C. At the temperature of 26°C. cercariae emerged from five snails within $2\frac{1}{2}$ hours and by the end of 24 hours emergence had been observed from all the snails in the group. Meanwhile, in the cabinet which was maintained at approximately 10°C., emergence was observed within $2\frac{1}{2}$ hours from seven snails and at the end of 24 hours parasites had emerged from eight out of the ten snails. This showed that cercariae emerged equally at 26°C. or at a temperature (10°C.) which was only slightly above the critical minimum. Further observations showed that emergence might occur throughout a wide range of intermediate temperatures and under conditions of rising or of falling temperatures. During all these observations there was no evidence of any significant differences in the numbers of parasites which emerged and at all temperatures encystment seemed to be normal.

B. THE EFFECT OF LIGHT ON THE EMERGENCE OF CERCARIAE.

Emergence which might be associated with the changed conditions of day and night has often been reported from observations on species of cercariae other than *F. hepatica*. Szidat (1929) noted the emergence of *Bilharziella polonica* at dusk or at night, a habit presumably denoting adaptation to the dusk-feeding habits of the wild ducks which act as

final hosts. Conversely, it has been shown by Faust and Hoffman (1934) and by Giovannola (1936) that the cercariae of *Schistosoma mansoni* emerge most readily under conditions of maximum sunlight or in bright artificial light. Rees, in 1948, summarised information on the periodicity of emergence of various species of cercaria and went on to show that *Cercariae purpurae* emerged from *Nucella lapillus* (L) predominantly in the light when the snails were exposed to alternating darkness and light. Continuous darkness appeared to inhibit emergence.

Our observations on the cercariae of *F. hepatica* showed that emergence occurred equally by day or by night; under conditions of full

TABLE I.

Illustrating the effect of variation of illumination on the emergence of the cercariae of F. hepatica.

Conditions during observation period of 24 hours	*Conditions during previous 24 hours*	*Number of snails*	*Number from which cercariae emerged*	*Total number of cercariae*
Darkness	Normal day–night illumination	100	49	676
Light	Normal day–night illumination	100	40	653
Darkness	Darkness	50	25	302
Light	Light	50	17	304
Light	Darkness	50	15	102
Darkness	Light	50	22	120

illumination and also in total darkness. There was no evidence of any difference in the numbers of cercariae which emerged at different times of day, although such quantitative estimates were difficult to obtain owing to the great variation in the numbers of cercariae which emerged from individual snails. To confirm that changes in illumination had no effect, 200 infected *L. truncatula* were divided into equal groups, snails from the first group (Group A) being placed individually in water in small glass tubes which were then kept at room temperatures in darkness for 24 hours. During this period a total of 676 cercariae emerged from 49 of the snails. Snails in the similar Group B were continuously illuminated for 24 hours, during which period a total of

653 cercariae emerged from 40 of the snails. This indicated that cercariae emerge equally under conditions of light or of darkness. The observation was continued by exposing 50 snails from Group A to a further 24 hours' darkness, during which 302 more cercariae emerged from 25 of the snails. The remaining 50 snails from Group A were taken from the darkness and exposed to continuous illumination for 24 hours, with the result that 102 cercariae emerged from 15 snails. These and other observations are tabulated in Table I, which confirms that alteration in the conditions of light and darkness had no effect on the emergence of the cercariae of *F. hepatica*.

Further observation showed that there was no relationship between the emergence of cercariae from the snails and any particular period of the day or night. Under laboratory conditions most cercariae appeared to emerge during the morning but this was found to be associated with the management of the snails under observation and not directly with the time of day. Each morning, the experimental snails were moved to fresh tubes of clean water, whereupon emergence usually followed within two or three hours. Ordinary laboratory routine resulted in most cercariae emerging during the morning but (as will be discussed later) the principal factor governing the emergence of the parasites was the immersion of the snail in fresh water. If snails were moved to fresh tubes in the late afternoon or at night cercariae emerged in numbers comparable with those observed earlier in the day.

C. THE EFFECT OF WATER IN INDUCING THE EMERGENCE OF CERCARIAE.

Experience showed that cercariae infecting field snails which had been brought to the laboratory for examination commonly emerged in very large numbers soon after the snails had been placed in clean water in the small glass tubes used for observation. Water acted as a primary stimulus to emergence, and in the laboratory we were able to show that even the small amount of water which accumulated when dry snails were sprayed with an atomiser was sufficient to induce emergence. This confirmed an observation of Walton (1918), who had shown that cercariae were released when aestivating snails on grass stems were damped with a little water.

Furthermore, from the study of snails in the field and in the laboratory it was soon apparent that water influenced the emergence of cercariae in another way. Careful observation of the maximum numbers of cercariae emerging from snails at the laboratory, and numerous estimations of the numbers of rediae and of cercariae found

in snails collected in the field or infected at the laboratory, strongly indicated that the large numbers of parasites which emerged from freshly collected field snails were considerably more than the average. Such numbers represented an accumulation which must have matured over a period of days or weeks but which had not emerged under field conditions. Yet in very many instances the snails had been collected from watery habitats and there seemed no reason why these mature cercariae should not have emerged. Some factor was evidently inhibiting emergence. Other laboratory observations showed the existence of a similar phenomenon. When freshly collected field snails were kept for observation in small glass tubes containing a few

TABLE II.

Showing that change of water induced the emergence of the cercariae of F. hepatica.

Group	*Conditions*	*Number of snails*	*Number from which cercariae emerged*
A	Snails left undisturbed	50	1
B	Snails returned to same water after mechanical disturbance	50	2
C	Snails placed in water previously occupied by other snails	50	6
D	Snails placed in fresh pond water ..	50	27

mls. of water, emergence was usually inhibited after the first 24 or 48 hours but could be induced, very quickly, by replacing the water in the tubes with fresh water. Filtered pond water of the type usually used in our culture dishes was most often added, but distilled water and ordinary tap water were equally effective. This secondary effect of water in inducing emergence was confirmed by observations on the colonies of snails infected at the laboratory. Here snails were kept in culture dishes, each with a mud slope and a small pool of water to which the snails had easy access and in which they were usually found. Yet it was rare to see evidence of emergence in undisturbed culture dishes, although when the snails were removed to other vessels for observation as many as 1,000 cercariae might emerge during the first 24 hours, this clearly indicating the accumulation of mature parasites over a period of weeks. Similarly, the addition of fresh water to the

previously undisturbed culture dish soon induced emergence from a high proportion of the snails.

Under more controlled conditions it was possible to establish that changing the water in which the snails live was an important factor in inducing emergence. For such an observation 200 snails, a high proportion of which was known to contain mature infections, were divided at random into four groups. As shown in Table II snails in Group A were left undisturbed as controls, while in the other groups they were mechanically disturbed (Group B), moved to water which had been occupied by other snails (Group C), or placed in fresh pond water (Group D). From an examination of Table II it is evident that the observed inhibition of emergence must be connected with previous occupation of the water by the snail itself or by another snail.

The effect of a change of water in inducing the emergence of cercariae had previously been observed by Taylor and Baylis (1930) when working with dermatitis-producing cercariae and later by Brackett (1940) and it was suggested that change in the temperature of the water in which the snail was immersed was instrumental in inducing emergence. With *F. hepatica* this did not appear to apply and we were able to repeat the observation recorded in Table II under conditions which avoided any possibility of a small difference of temperature in the water into which the snails were moved.

It was clear that the conditions developing in the small volume of water in which a snail was confined tended to restrict emergence. Preliminary observations had shown that emergence could be induced by fresh pond water, distilled water or ordinary tap water, so that it was not clear what were the precise physical or chemical factors involved. Various possibilities were accordingly investigated.

The effect of depletion of oxygen in the water containing the snail.

To test this possibility, tap water was boiled continuously for three hours, then sealed and cooled rapidly in a refrigerator until its temperature reached 26°C. The emergence which occurred in this water was then compared with that which occurred in unboiled water at the same temperature and under the same conditions. Two groups, each consisting of ten snails which contained cercariae ready for emergence, were used in the observation, snails being placed individually into small glass tubes, which were rapidly filled to the brim, with boiled or with unboiled water, tightly corked and sealed with paraffin wax. Cercariae were observed to emerge from snails in both

groups and there was no apparent difference in the times of emergence or in the numbers of cercariae produced.

The effect of an increase in the concentration of carbon-dioxide.

Preliminary observations on medium sized uninfected *L. truncatula* suggested that a vol./vol. concentration of 20% carbon-dioxide in distilled water was the maximum in which survival of a reasonable proportion of the snails could be assured. The effect of this concentration on the emergence of cercariae was accordingly observed.

The infected snails were thoroughly cleaned of adhering débris and algal growth and each was confined in a small glass tube completely filled either with distilled water or with water containing 20% of carbon-dioxide. After filling each tube a well fitting cork was inserted and sealed with melted paraffin wax. Observation showed that this concentration of carbon-dioxide did not inhibit emergence since cercariae emerged from 14 out of 15 snails in plain distilled water, while emergence was noted from 14 out of 18 snails in the water containing 20% of carbon-dioxide. There was thus no difference in the final result between the groups. Nevertheless, there was some indication that a delay in emergence occurred in the snails exposed to a high concentration of carbon-dioxide. Whereas, after only seven hours' observation, emergence had occurred from 12 (out of 15) snails in distilled water, only seven (out of 18) snails showed emergence in the group exposed to 20% of carbon-dioxide. It appeared that an increase in the level of carbon-dioxide in the water might limit emergence but it did not appear to be the major factor involved.

The effect on emergence of a change in the hydrogen-ion concentration of the water containing the snails.

Excretion into the water of waste products of metabolism of the snails might affect the hydrogen-ion concentration in turn influencing the emergence of cercariae. Such a possibility offered an explanation of our experimental observations on the inhibition of emergence shown when snails were confined for considerable periods in small sealed tubes.

Bauman et al (1948) found that an alkalinity corresponding to a hydrogen-ion concentration of 7.6 was a critical factor in the release of the cercariae of *Schistosoma japonicum* from the snail *Oncomelania quadrasi*. These workers used waters from various natural sources, the hydrogen-ion concentrations ranging from 6.4 to 7.6. In their

experience cercariae did not emerge at a *p*H lower than 7.2, although a few were released at this degree of alkalinity. During the observations, by far the greatest numbers were shed at *p*H 7.6, although the effect of water of an alkalinity greater than *p*H 7.6 was not ascertained.

In our initial observations it was decided to investigate the limits of acidity within which the snail itself could live, not necessarily for prolonged periods but at least for the duration of the experimental period. Evidence from the field, possibly owing to the pronounced diurnal variability shown by natural waters (Boycott, 1936), was difficult to obtain. Some observers suggested that the snail preferred an environment on the alkaline side of neutrality. Thus Atkins and Lebour (1924) found the greatest abundance of *L. truncatula* in an area of "highly calcareous water" with a *p*H range of 7.8 to 8.0; Bertram (1939) recorded a hydrogen-ion concentration range of 7.3 to 7.7 for this species on certain of the inner Hebridean Islands, while Hatzky (1940) stated that the optimum *p*H for *L. truncatula* was between 7.0 and 8.0. On the other hand, Walton and Wright (1926), in an attempt to correlate the distribution of Limnaeid snails with the hydrogen-ion concentrations of their habitats in North Wales, came to the conclusion that *L. truncatula* was found in water which gave *p*H readings of 6.4 to 7.8, while the soil where the molluscs lived indicated 5.8. Okland (1936) observed *L. truncatula* in water of *p*H 5.6 to 8.6, and Peters (1938) found the species in habitats with *p*H ranges of 6.9 to 8.2.

In our laboratory observations distilled water was adjusted to the required hydrogen-ion concentration with a sodium phosphate buffer. Initially, 200 medium-sized (0.4 cms. to 0.5 cms.) uninfected snails were divided into four groups and the mortality in water at buffered hydrogen-ion concentrations of 8.0, 7.0 and 5.5, compared with that in ordinary pond water with a hydrogen-ion concentration of approximately 7.0. Viability of the snails at 24, 48 and 96 hours was observed and it was seen that water buffered to a hydrogen-ion concentration of 5.5 proved relatively unsuitable for the snails, 25 out of 50 being dead after 96 hours, as compared with 3 out of 50 in the pond water. Alkaline water appeared much more favourable to survival; and deaths, as in the pond water to which the snails were accustomed, were very few. Nevertheless, the majority of snails, even at a hydrogen-ion concentration of 5.5, were still alive after 48 hours. Since the period of time necessary to observe the emergence of cercariae was unlikely to exceed 48 hours it was decided to observe

the effect on emergence of cercariae of different hydrogen-ion concentrations within the range of 5.5 to 8.5.

Forty *L. truncatula*, from each of which cercariae were known to have emerged, were divided into two groups and, after carefully cleaning the shells of adhering débris, each snail was placed in a 15 ml. test-tube. Distilled water, buffered with $\frac{M}{500}$ sodium phosphate to a hydrogen-ion concentration of 5.5, was added to twenty of the tubes, while water buffered to a hydrogen-ion concentration of 8.5 was added to the others. The tubes were then tightly corked and sealed with paraffin wax. Within two hours of the commencement of the observation cercariae were observed to be emerging from fourteen of the snails in water at a hydrogen-ion concentration of 5.5 and from fifteen of the snails at a hydrogen-ion concentration of 8.5. At the end of seventeen hours cercariae had emerged from eighteen snails in the acid water and from twenty snails in the alkaline water. At this time the hydrogen-ion concentration of the water in the tubes was checked. It was found that water originally buffered to 5.5 was now at 5.7 to 5.8, while that originally at 8.5 indicated a hydrogen-ion concentration of 8.4. This showed that the hydrogen-ion concentrations in the tubes had not varied greatly throughout the experimental period.

The observation was repeated using a sodium phosphate buffer at $\frac{M}{250}$, with similar results. It was concluded that the cercariae of *F. hepatica* emerged equally into acid or into alkaline water.

Discussion.

During our observations on the cercariae of *F. hepatica* and the snail *Limnaea truncatula* it appeared that emergence did not occur below a temperature of 9°C. Apart from the existence of this critical temperature the presence of water was the only clearly indicated factor influencing emergence. When snails which had been resting under dry conditions were immersed in water or when the snails were sprayed with a small quantity of water, the great majority of mature cercariae emerged. Water induced emergence within a very few hours and to this extent the emergence of *F. hepatica* could be assessed in simple physical terms. It must be remembered, however, that the snail, which is essentially an amphibious animal, spends a considerable proportion of its time immersed in shallow water, and our observations showed that under such conditions mature cercariae did not always emerge. Their emergence was induced under laboratory conditions by

changing the water in which the snail was resting. Fresh pond water of the type to which the snails were accustomed, tap water or ordinary distilled water were all effective. Although changing the water in which the snails rested induced emergence, we were unable to show in what respect the fresh water differed from the water which it replaced. Above the temperature critical for emergence heat had no apparent effect and the cercariae emerged indifferently in light or darkness, in water depleted of oxygen or in which the carbon-dioxide was increased, and within a hydrogen-ion concentration range of from 5.5 to 8.5. There was, however, some evidence that an increase in the amount of carbon-dioxide in the water delayed emergence although it did not affect the final result.

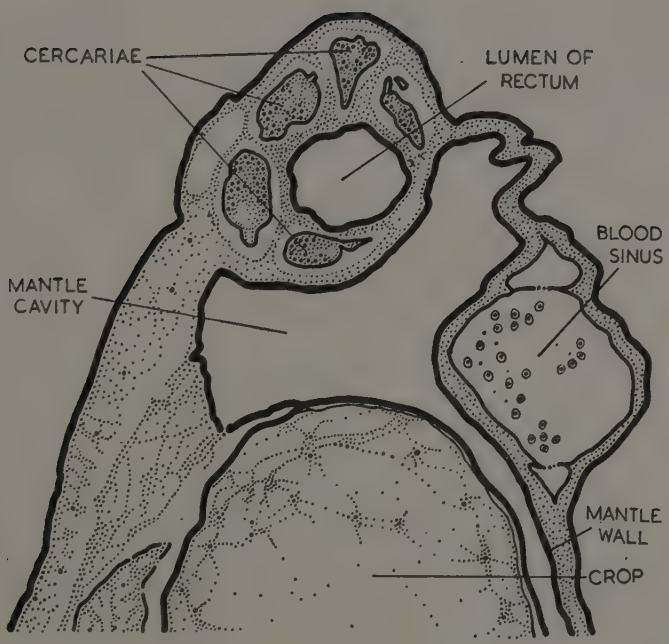
It was noted that changing the water in which the snails rested tended not only to induce emergence but seemed also to increase the activity of the snails themselves. Consideration of the exact mode of emergence of cercariae from a snail suggests that stimulation of the resting snail may be the principal factor causing emergence. When a snail, containing mature parasites, is taken from a mud slope or from the shallow water of its habitat large numbers of cercariae may be seen through the shell of the body whorl moving slowly within the cavity surrounding the alimentary tract of the snail. If the snail is placed in a small amount of clean water the cercariae soon show signs of greater activity, which seems to be associated with such movements of the snail as the extrusion of head and foot and the stretching and contraction of the mantle wall. After a short time the activity of the cercariae becomes very pronounced and they begin to congregate in the perivisceral space surrounding the distal part of the gut of the snail. Shortly before emergence actually occurs, an area immediately contiguous with the anus of the snail becomes tumid and assumes a teat-like appearance. Cercariae can now be seen within the perivisceral space (Fig. 1) surrounding the rectum and moving towards the exterior. As the pneumostone of the snail closes cercariae begin to be extruded through the teat-like process. Observation of the process strongly suggests that in its later stages emergence of the cercariae is predominantly passive. Each cercaria of a series appears to be extruded with considerable force and seems comparatively inactive when newly emerged. After emergence the parasites swim slowly away from the snail, and on several occasions individual cercariae were observed to be sucked into the mantle cavity by an inhalation of water immediately following the emergence.

Since observation suggested that the cercariae took little active part in the process of emergence it is necessary to consider what physical and physiological processes in the snail may assist the process.

Figure 1, which illustrates a transverse section through *L. truncatula*, shows parasites within the perivisceral space and indicates the means by which the cercariae may be caused to emerge. It will be seen that the cercariae lie in comparatively restricted spaces surrounding the terminal part of the gut and that the mantle cavity is contiguous. As already indicated, emergence of the cercariae is associated with activity on the part of the snail and it is significant that the pneumostome appears to be closed at the actual moment of emergence. There is evidence (Van Someren, 1946) that snails at rest are able to adjust their oxygen consumption to the oxygen tension of the surrounding water and that a snail may not use the respiratory mechanism of the mantle cavity but rely on a shallow type of respiration using oxygen already in the cavity. Activity on the part of the snail will be associated with greatly augmented movements of the wall of the mantle cavity. Pressure set up in the mantle cavity while the mantle aperture is closed causes pressure on the perivisceral spaces which contain the emerging cercariae and under suitable conditions aids their progress towards the exterior. Such an explanation of the mechanism of emergence received support from the observation that a resting snail, when dropped into a vessel of water, sometimes emits a cloud of cercariae apparently as a direct result of convulsive movement of the walls of the mantle cavity.

The fact that emergence is related to exceptional activity of the mantle, which in turn is apparently stimulated when the snail comes in contact with fresh water, has an important bearing on the epidemiology of the disease of Fascioliasis, for it may be inferred that under field conditions rainfall or the actual movement of surface water will lead to increased activity of the snail and to a mass emergence of cercariae. Consideration of these circumstances under which cercariae emerge suggests also the way in which the parasites become accessible to grazing stock. Cercariae emerge most readily and in the greatest numbers into shallow temporary water which has collected as the result of rainfall or local flooding. Recession of flood water, absorption of rain or the drying out of dew leave the encysted cercariae in particularly favourable conditions for ingestion by the definitive host.

The amphibious nature of *L. truncatula*, its preference for habitats which are subject to alternate flooding and drying and the mechanism



Fasciola hepatica in *Limnaea truncatula*.

Fig. 1.—Transverse section of snail showing distribution of cercariae round the rectum.

of emergence of the cercariae under conditions of temporary flooding all contribute to the perfection of the host-parasite relationship and illustrate the great efficiency of *L. truncatula* as a vector.

Summary.

1. Observations on *Fasciola hepatica* in *Limnaea truncatula* under laboratory conditions showed that the cercariae did not emerge at temperatures below 9°C. Above 9°C. emergence proceeded indiscriminately up to the limit of experimental observation (26°C.).

2. Experience showed that emergence occurred in the light or in darkness while changes in the hydrogen-ion concentration of the water containing the snails (within the range 5.5 to 8.5) had no effect on emergence. There was some evidence that an increase of carbon-dioxide in the water slowed but did not inhibit emergence but depletion of oxygen had no apparent effect.

3. In the laboratory, emergence could be induced by removing snails from dry habitats and immersing them in water or by removing snails from watery habitats and placing them in fresh water. We were unable to determine the exact physical or chemical factors acting as a stimulus under such conditions.

4. Observation suggests that the emergence of the cercariae of *F. hepatica* is largely passive and that the parasites are expelled from the body cavity of the snail as a result of unusual activity of the mantle causing increased pressure on the terminal part of the perivisceral space.

5. In the field temporary flooding of the habitat of the snail leads to circumstances favourable both for the emergence of cercariae and for their encystment under conditions likely to favour transmission to the definitive host.

Acknowledgments.

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On the Transference of Adult *Paramphistomum hiberniae* Willmott, 1950 from One Definitive Host to Another of the Same Species.

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Owing to the comparative rarity of Paramphistomes in Britain the difficulties of attempting to work out the life cycle are very great. Up to the present the problem has been approached from two angles; firstly, where an infected animal has been found at the abattoir, and finally traced back to its farm of origin, extensive collection of snails has been carried out at the assumed site of infestation; secondly, where it has been possible to collect large numbers of adult worms from slaughtered animals, these have been picked out of the rumen and placed in dishes containing boiled tap water for several hours, in which medium oviposition takes place rapidly. These eggs have been collected and cultured, and laboratory-bred snails have been exposed to the miracidia, but so far no infection has taken place. This has of necessity been sporadic and no concentrated systematic attempts at infection over long periods have been possible.

It was therefore suggested to the authors by Professor R. T. Leiper that it might be possible to transfer adults collected from one host into the rumen of an experimental animal and so ensure a constant supply of eggs which would be collected from the faeces by sedimentation or similar concentration methods.

For this purpose it was essential to determine the length of time these parasites could be maintained alive outside the body of the host and the best method of transporting them from the abattoir where they were collected, to the field station where they could be given to an experimental animal, which involved a period of delay of at least two days.

The experiment was of a rough and ready nature as there were no facilities available for aseptic techniques, preparation of culture media, elaborate incubators and the like. Deschiens and Pick (1948) in their work on *Watsonius watsoni* have shown that by using artificial culture

media they were able to keep the parasites alive for periods up to eighteen days. A comparison of these results is interesting, for although *Watsonius watsoni* and *Paramphistomum hiberniae* are closely related genera, their hosts and habitats are very different. It is hoped to extend the work on *P. hiberniae* as soon as the opportunity offers.

For the purpose of these experiments, the parasites were assumed to be still alive while movement could be induced, either by warming gently those kept at room temperature or cooling those kept at approximately 35°C. Rumen fluid was obtained by straining rumen content through a coarse sieve, boiling the fluid obtained for 10 minutes and allowing it to cool either to room temperature or to 35°C. Amphistomes were removed from the rumen within one half hour of the animal being felled ; they were divided into four groups and treated as follows :

Group 1 was put into boiled tap water at room temperature.

Group 2 was put into saline at room temperature.

Group 3 was put into rumen fluid at room temperature.

Group 4 was put into rumen fluid at 35°C. in a thermos flask.

Later group 4 was split into two, one section being kept in the thermos flask and the other, group 4a, being transferred to fresh, boiled rumen fluid in a small glass screw-capped container which was strapped by means of plaster to the body of one of the authors in order to maintain a constant temperature. All groups, 1–4, were observed during the first day at intervals of a few hours, groups 4 and 4a were subsequently examined every twelve hours.

A few eggs were found in the fluid each time it was changed and these appeared to be quite normal. Worms were removed at $5\frac{1}{2}$ days, 8 days and 9 days and fixed for sectioning in order to study any change that may have taken place in the body. Several of the worms were dead on the ninth day. These were discarded and only those which still responded to stimulation were taken for fixation. These were fixed in 10% formalin, embedded in paraffin wax and sections were cut at 10μ, stained with Ehrlich's acid haematoxylin, and counterstained with Eosin. Some were shown at a laboratory meeting of the Royal Society of Tropical Medicine and Hygiene. (Willmott, 1951).

In view of the small scale of the experiment and the rather crude techniques used it would be unwise to generalise at this stage. It is, however, obvious that up to nine days after removal from the host very little degeneration has taken place. From this one may infer that had it been possible to maintain the Paramphistomes in rumen

fluid free from bacterial decomposition their lives might have been further prolonged. Unfortunately, the supply of prepared rumen fluid was exhausted by the eighth day, and no further changes of the medium were possible.

From preliminary histological studies it appears that the vitellaria are the first organs to be affected; this is confirmed in the nine-day specimens by the presence in the proximal part of the uterus, of oocytes

Group No.	*Medium*	*Temperature*	*Length of life*	*Remarks*
1	Boiled tap water	Room	5 hours	Showed no tendency to attach to one another. Very little movement at any time.
2	Saline	Room	6 hours	As for group 1.
3	Rumen fluid	Room	8 hours	Slight tendency to attach to one another.
4	Rumen fluid	Thermos flask 35°C.	32 hours	Attached themselves to one another within 5 hours. After 22 hours fluid had dropped to room temperature. The fluid was warmed again and they were left in the thermos flask and lived another 10 hours, becoming gradually less active.
4a	Rumen fluid	Body heat approx. 35°C.	9 days	This group was removed from group 4 after 22 hours, put into fresh rumen fluid at 35°C. in a small screw-capped container and kept at body temperature. These attached themselves rapidly to one another and one became attached to the wall of the container and remained there for 3 days in spite of changing the rumen fluid every 36 hours. Before changing the fluid the parasites were rinsed in boiled tap water at 35°C.

around which no shell has formed, although eggs with shells are present in the distal portion. By the ninth day the ovary and testes show signs of some degeneration, but the general body muscles, the pharynx, acetabulum, genital atrium, gut, caeca, Mehlis' gland and the excretory bladder still appear to be quite normal and no very marked reduction in size has occurred.

From the above experiments it can be seen that it is quite possible to remove adult worms from the rumen of a slaughtered animal, and

to transport them a considerable distance in a viable condition, provided that they are kept at a constant temperature and in rumen fluid.

As a result of this preliminary work, two calves at the London School of Hygiene and Tropical Medicine Field Station, Winches Farm, St. Albans, were drenched with rumen fluid containing living Paramphistomes. Examination of the faeces before the beginning of the experiment showed no Paramphistome infection. A fortnight after dosing, Paramphistome eggs in small numbers were found in the faeces.

Further examination of faecal samples at intervals of approximately four weeks showed that the experimental animals were retaining the infection. At the end of three months there had been an increase in the number of eggs found in the samples, indicating that not all the parasites introduced into the host had been mature.

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On the Possibility of *Panagrellus silusiae* Parasitizing the Mouse under Experimental Conditions.

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In the course of evolution of the Nematoda, the step from a free-living existence to a parasitic one has, in all probability, been taken several times. Many typically free-living species of nematodes have occasionally been found living within the bodies of other animals. Chandler (1928) reports the occurrence of *Diploscapter coronata*, normally an inhabitant of soil and sewage beds, within the stomachs of nine people suffering from complete or almost complete lack of hydrochloric acid in the stomach. Re-examination four days later showed the worms to be still present and hence it seems probable that they were definitely established. Yokogawa (1936) reports the infection of the urino-genital system of a Japanese woman by the same worm. *Rhabditis pellio*, parasitic in the coelom of earthworms as a larva and living on the decaying remains of earthworms after attaining maturity, has occasionally been found living in the human vagina.

It was decided therefore to take a typically free-living nematode and investigate its physiology with the object of determining the possibility of its survival within the alimentary canal of a mammal. The species chosen was *Panagrellus silusiae* (de Man, 1913) Goodey, 1945, a worm first discovered as an inhabitant of so-called " beer felts " in Germany, and now used extensively by aquarists as food for fish. The male worms are 1.8 mm. to 2.0 mm. long and the females 2.5 mm. to 2.7 mm. long. The tail tapers to a fine point in both sexes and the cuticle shows longitudinal striations. The mouth is 13–15 μ deep and there are three projecting lips. The male possesses two spicules, both approximately 56 μ in length with the proximal end hooked. The accessory piece or gubernaculum is about half the length of the spicule. Seven pairs of papillae are present in the male, two pre-anal, one on a level with the anus and four post-anal. In the female the ovary is single and anterior and the uterus has a post-vulval projection. The worm is viviparous. It is readily cultured on oatmeal mixed with water and boiled to a thin porridge-like consistency.

Investigation of the culture medium after culturing P. silusiae *on oatmeal porridge for 7 days.*

A dozen Petri dishes were filled with oatmeal porridge and each inoculated with a living culture of *P. silusiae*. They were kept at a constant temperature of 60°F. for 7 days. During that time the cultures became liquified and developed a strong smell of acetic acid. At the end of the period the *p*H of the medium was tested by means of a *p*H meter and was found to be 3.84 at 18.5°C. The medium was distilled and the distillate tested for acetic acid. Acetic acid was found to be present.

TABLE I.

p*H*	*No. of worms alive in the* 10 *dishes*			
	After 3 *hours*	*After* 6 *hours*	*After* 9 *hours*	*After* 12 *hours*
1.0	98	90	85	82
7.5	98	97	97	96
8.0	95	93	82	61
8.5	80	60	40	12
9.0	70	43	0	0
9.5	60	31	2	0
10.0	30	3	1	0

It seemed reasonable to suppose that considering the low *p*H produced by their own metabolic activities, or possibly by the selective influence exerted by the worms on the bacterial decomposition of the porridge, the nematodes should be able to stand the acidity of the mammalian stomach. However, it was decided to investigate the *p*H tolerance range of *P. silusiae* more thoroughly.

Investigation of p*H tolerance of* P. silusiae.

A series of phosphate buffer solutions was made up covering the *p*H range 4.0–9.0 and a series of hydrochloric acid buffers for the range 1.0–4.0 ; above 9.0 to a *p*H of 10.0, carbonate buffers were used.

These solutions were placed in covered cavity dishes, ten dishes being used for each *p*H value. The *p*H interval between the various solutions was 0.5. In each dish ten mature worms, taken by a pipette from a very dilute culture, were placed. The dishes were examined at three-hourly intervals and the results are summarised in Table I.

The results suggest that the *p*H tolerance of *P. silusiae* would allow it to survive in the mammalian alimentary tract, in so far as that particular factor is concerned. It is rather interesting to find that *P. silusiae* is as tolerant of acid conditions as the rather similar vinegar eelworm, *Turbatrix aceti* (Müller, 1783) Peters, 1927, but less tolerant of alkaline conditions. Peters (1928) gives a *p*H range of 1.6–11 for *T. aceti*, whereas these experiments appear to give a range of *p*H 1.0–8.5 for *P. silusiae*.

TABLE II.

Medium	*No. of worms alive in* 10 *dishes* (*Larval counts in parenthesis*)				
	After 3 *hrs.*	*After* 6 *hrs.*	*After* 9 *hrs.*	*After* 12 *hrs.*	*After* 24 *hrs.*
Active gastric ..	98 (96)	97 (94)	96 (93)	95 (91)	90 (86)
Boiled gastric ..	97 (97)	96 (95)	95 (92)	93 (90)	91 (88)
Active pancreatic	80 (60)	80 (3)	31 (0)	3 (0)	0 (0)
Boiled pancreatic	82 (72)	80 (12)	60 (4)	8 (0)	1 (0)

Effect of digestive enzymes on P. silusiae.

Preparations of gastric juices and pancreatic juices, free from any added chemicals which might be injurious to the nematodes, were used for the experiments. As in the previous experiments, the cultures were incubated at approximately body temperature (100°F.) and a hundred worms distributed throughout ten cavity dishes were used.

A normal culture of *P. silusiae* contains numerous viviparous females and rather less numerous males and larvae. In the above experiments full grown males and females had been used. It was decided to repeat the experiment to show the effect of digestive enzymes on larvae. The results are given in Table II, the figures for larvae being in parenthesis after the counts for adults.

The results suggest that *P. silusiae* is perfectly tolerant of the enzyme action of the gastric juice, but that it is rapidly destroyed by pancreatic juice. The high *p*H of pancreatic juice appears to be responsible for the death of the worms in the first place, although later their body tissues are dissolved, for after 12 hours in pancreatic juice only the cuticle is left. In boiled pancreatic juice the animals die but traces of their internal structure can be seen even after 24 hours. The larvae show similar responses to those shown by the adults, except that they are even less tolerant of the pancreatic juice.

TABLE III.

Temp.	*No. of worms alive in* 10 *dishes* (*Larval counts in parenthesis*)				
	After 1 *day*	*After* 2 *days*	*After* 3 *days*	*After* 4 *days*	*After* 5 *days*
30°C.	98 (96)	94 (93)	90 (90)	85 (84)	81 (79)
35°C.	98 (95)	93 (91)	89 (86)	86 (83)	82 (79)
37°C.	98 (97)	95 (92)	90 (82)	81 (79)	73 (63)
40°C.	82 (57)	61 (43)	58 (31)	50 (22)	43 (6)
45°C.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Effect of temperature on P. silusiae.

Although in the last two experiments the worms survived a body temperature of 100°F. for 24 hours, it seemed desirable to investigate the effect of temperature on the worms more fully. *P. silusiae* thrives in a medium of agar containing 0.5 gm. of peptone and 0.5 gm. of glucose per 100 c.c. of 2% solution of agar. This is a more suitable medium than oatmeal porridge in which to observe the worm. Accordingly 10 cavity dishes each containing 10 worms were set up and incubated at 30°C.; 10 at 35°C.; 10 at 37°C.; 10 at 40°C.; and 10 at 45°C. The experiment was duplicated using larvae and the results are given in Table III.

It would appear from the results that, although a reasonable number of the adult nematodes could survive the conditions of temperature in the mammalian alimentary tract, the larvae are slightly

less tolerant and the completion of the life cycle within the alimentary tract seems an unlikely event. In this respect, however, *P. silusiae* seems more tolerant of expected alimentary tract conditions than *T. aceti* which Peters (1928) found to perish within 9 days at 37°C., whereas the writer has found that *P. silusiae* continues to survive and reproduce for a long time at that temperature.

Effect of lack of oxygen on P. silusiae.

Using the same culture medium as in the last series of experiments, 10 cavity dishes containing 10 worms each were set up and placed in a chamber of pure nitrogen. A similar series of dishes was prepared and the surface of the culture liquid covered with Nujol, thereby excluding the air, but obviously retaining any carbon-dioxide produced in the respiration of the worms. The dishes were examined after 6, 12 and 24 hours and the results are set out in Table IV.

Table IV.

	No. of worms alive out of 100		
	After 6 *hours*	*After* 12 *hours*	*After* 24 *hours*
In Nitrogen ..	98	97	97
Under Nujol ..	96	70	2

It would appear that *P. silusiae* can exist for at least a limited period anaerobically, provided that the carbon-dioxide produced by its own respiration is not allowed to accumulate.

The above experiments seem to suggest that *P. silusiae* should be able to survive the conditions of temperature, *p*H value and oxygen concentration occurring in the anterior part of the alimentary canal of mammals. It was therefore decided to test directly whether such survival was possible.

Effect of feeding P. silusiae *to mice.*

A small amount of oatmeal culture containing abundant nematodes was mixed with oats and fed to mice which had not previously eaten for ten hours. The oats were readily eaten and immediately after the meal four of the mice were killed with a sharp blow at the back

of the neck and their stomach contents examined. All four contained numerous *P. silusiae* in the stomach. The worms are readily distinguished from nematodes normally parasitic in mice, but the particular mice in question belonged to a batch previously treated with hexylresorcinol and known to be free from helminths.

After three hours a further four mice were killed and their stomach contents examined. Living *P. silusiae* were found, although not as abundantly as before.

After six hours a further search was made which yielded few living *P. silusiae,* although numerous dead ones were found in the caecum.

The remaining four mice were sacrificed after twelve hours, having been fed in the meantime. No living worms were found in the stomach although there were abundant dead worms in the caecum.

It would appear, therefore, that although *P. silusiae* can tolerate conditions in the stomach of the mouse, when fed to mice it gets carried back into the intestine where conditions are unfavourable. It is significant that although the worms seem to disappear from the stomach, few dead ones are found in that organ. *P. silusiae,* therefore, seems unlikely ever to become a facultative parasite of the alimentary canal of mammals. A similar conclusion was reached by Peters (1928) for *T. aceti,* although Wiel in his " Diätetischen Kochbuch für Gesunde und Kranke " (1881, p. 178) gives clinical evidence of the vinegar eelworm causing gastric complaints in one household of his medical practice. As Peters points out, however, these symptoms are purely gastric and might well be produced by the continuous introduction of worms into the stomach, at each successive intake of infected vinegar. Thus there appears to be complete agreement between the results of these experiments with *P. silusiae* and what has hitherto been observed for *T. aceti.*

Infection of urino-genital system of a female mouse by P. silusiae.

The experiment of feeding *P. silusiae* to mice was repeated several times and consistently yielded the results described above. In one cage used for these experiments a female mouse was noticed with the vulval region inflamed. The mouse showed a strong tendency to lick and scratch this region, which seemed to cause it considerable irritation. The vagina was douched out with water from a pipette and the liquid so obtained was found to contain numerous *P. silusiae.* The mouse was kept under close observation and yielded several *P. silusiae* from vaginal washings for three days. After that period the irritation seemed to have disappeared, vaginal washings showed

no further nematodes and on killing the mouse and examining the urino-genital tract it was found to be free from nematodes.

As a result of this accidental establishment of *P. silusiae* in the female urino-genital tract of the mouse, experiments were performed to test the possibility of its repetition. The vulval region of ten mice was smeared with a culture containing *P. silusiae*. After such treatment, two of the mice tended to lick that region and probably removed the bulk of the culture material; the remaining mice paid no attention to the smeared material.

The following day two of the mice had the same symptoms as the accidentally infected mouse, namely, scratching and licking of the vulval region. The symptoms continued for three days and then disappeared. During that period no vaginal douches were taken, as it was possible that in the accidental establishment of *P. silusiae* in the urino-genital tract, as first described, the washings caused the worms to disappear. Repetition of the experiments has shown that *P. silusiae* will enter the vagina of the mouse and remain there for a limited period, during which time it appears to cause some irritation. Its permanent establishment in the female urino-genital tract of the mouse has yet to be proved.

Discussion.

Records of normally free-living nematodes establishing themselves within the urino-genital tract of mammals are already to be found in the literature. Reference has been made to *Diploscapter coronata* and *Rhabditis pellio* in this connexion. It is of interest also that *T. aceti*, more closely related to *P. silusiae* than either of the other two nematodes, has been reported as occurring in the human urino-genital tract. Stiles (1902) reports an infection of the bladder by the vinegar eelworm in which the nematodes appeared in the urine. It would appear, therefore, that *P. silusiae* has rather more chance of establishing itself in the mammalian urino-genital tract than in the alimentary canal. Whether such establishment could be permanent remains yet to be seen.

Summary.

1. *Panagrellus silusiae* was cultured in various media to determine the possibility of establishing it as a facultative parasite in the mammalian body. It was found to tolerate *p*H values of its environment between 1.0–8.5.

2. It survived for 24 hours in gastric juice but was rapidly killed by pancreatic juice. In both preparations larval mortality was higher than that of adults.

3. *P. silusiae* withstood environmental temperatures up to 37°C. At 40°C. death did not occur within 24 hours.

4. *P. silusiae* could survive without oxygen for at least 24 hours provided that the carbon-dioxide produced by its own respiration was not allowed to accumulate.

5. When experimentally fed to mice, the nematode remained alive for a short time in the stomach but was killed as it passed with the semi-digested food into the intestine.

6. *P. silusiae* was shown to invade the vagina when in the vicinity of that organ and remain there for about three days, during which time it caused a certain amount of irritation.

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The Helminth Parasites of Domesticated Birds in Mid Wales.

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The study of the helminth infestations of domesticated birds in Britain has received scant attention in recent years, although earlier workers, including Clapham (1935, 1936, 1937, 1938a, 1938b, 1940), Morgan and Wilson (1938, 1939) and Taylor (1938), reported the occurrence of many species of parasitic worms in British poultry and game birds.

Most of the common helminth parasites of domesticated birds have at various times been incriminated as causal or associated factors in the production of disease and death, but little is known about the real pathogenicity of many of them or about the conditions under which infestations reach chronic or fatal levels. Other questions on which no information is available include the effect of multiple infestations with two or more species, and the possible inter-relationship between helminth infestation and diseases due to other causes.

Previous accounts of the importance of infestations with helminth parasites as factors leading to ill-health and death in domesticated birds have been concerned almost wholly with the helminths of domestic fowls. It was stated in the Report of the Poultry Technical Committee for Great Britain (1938) that worm infestation was one of the three most frequently diagnosed causes of death in domestic fowls, and it was emphasized that there was need for more intensive research on the effect of helminth infestations on the growth, health and productivity of fowls. The difficulty, mentioned in the Report, of accurately diagnosing helminthiasis was further emphasized by Taylor (1938), who stated that of the 150–200 helminth species known to occur in domesticated birds only two caused serious diseases which could be diagnosed with certainty—*Syngamus trachea*, the gape worm of fowls, and *Amidostomum anseris*, the gizzard worm of geese. Although it was probable that a large number of worms in any one bird was harmful, there was no real guide for diagnosis as usually there were no characteristic symptoms of disease, and at autopsy no characteristic lesions.

A more recent investigation into the causes of death of domestic fowls was that of Hoffman and Stover (1942), who analysed the results of 30,000 autopsies performed between 1930 and 1940 on fowls in the U.S.A., and found that 6% of the total deaths had been ascribed to infestation with intestinal nematodes, 1.5% to cestodes and 1.0% to gizzard worms. Intestinal nematodes, cestodes and gizzard worms were placed 3rd, 14th and 18th respectively, in a list of all causes of death arranged in order of frequency. Infestation with intestinal nematodes was most serious in birds between the ages of 7 months and 2 years. In birds aged 7 to 12 months it was responsible for 11.3% of the deaths, and in birds aged 1 to 2 years, for 10.8%, being in the latter age-group the most frequently diagnosed cause of death. Cestode infestation reached its maximum importance in birds 3 to 6 months old; it accounted for 2.9% of the deaths and was 7th in the order of frequency of the various causes of deaths. Gizzard worm infestation was most serious in birds between 1 and 2 years old; it caused 3.2% of the total number of deaths and was 10th in the list of causes of deaths.

The survey described below was carried out in order to obtain accurate information concerning the level of infestations in domestic birds in Mid Wales so that the knowledge gained would serve as a basis for further research on the pathogenicity of helminth infestations in poultry, and on the relationship between helminthiasis and other diseases. The data presented were obtained by the examination of the lower digestive tracts (duodenum to rectum) of 293 domestic fowls, 20 domestic turkeys, 30 domestic geese, 47 domestic ducks and 10 muscovy ducks during 1946 and 1947.*

Intestines of birds killed for human consumption were obtained from the University College of Wales Farm, Clarach, Aberystwyth (Source "A"), a commercial poultry farm near Aberystwyth (Source "B") and 27 mixed farms and small-holdings within a radius of 8 miles of Aberystwyth (Source "C"). In addition, the carcases of casualty and diseased birds sent to the Veterinary Investigation Officer, Aberystwyth, for autopsy were examined for helminth parasites (Source "D").

All parasites recovered, with the exception of the cestodes *Davainea proglottina* and *Amoebotaenia sphenoides*, were carefully counted, male and female nematodes being counted separately. The numbers of

*Detailed figures of the individual infestations of all intestines examined are available for consultation in the Library of the Department of Agriculture (Animal Husbandry), University College of Wales, Aberystwyth.

the two cestode species were estimated by a method similar to that used by Taylor (1933), as these small worms readily break up into separate segments on the death of the host.

The Helminth Parasites of the Domestic Fowl (*Gallus gallus dom.* (L.)).

THE SPECIES AND NUMBERS OF PARASITES RECOVERED.

The intestines of 267 adult fowls (birds over 12 weeks old) were examined; 243 (91%) were found to be infested with one or more species of helminth parasites. The total number of worms per infested bird varied between 1 and 3,139 and the percentage numbers of birds infested with 1–100 worms, 101–500, 501–1,000 and over 1,000 were respectively 46.8, 32.6, 7.5 and 4.1. The average infestation per bird was 240.6 worms of 2.42 species.

The intestines of 26 chickens between 2 and 12 weeks old which had been sent to the Veterinary Investigation Officer for autopsy were also examined; 14 were infested with from 1 to 3,829 worms. The average infestation per bird was 499.9 worms of 2.79 species.

The species of parasites found in domestic fowls were as follows :—

NEMATODES.

**Heterakis gallinae* (Gmelin, 1790).
Ascaridia galli (Schrank, 1788).
Capillaria caudinflata (Molin, 1858). Syn. *Capillaria longicollis auctt.*
**Capillaria collaris* (v. Linstow, 1873). Syn. *Capillaria retusa* (Railliet, 1895).
**Capillaria dujardini* Travassos, 1914. Syn. *Capillaria columbae auctt.*
Trichostrongylus tenuis (Mehlis, 1846).

CESTODES.

Davainea proglottina (Davaine, 1860).
Amoebotaenia sphenoides (Railliet, 1892).
Raillietina cesticillus (Molin, 1858).
Dilepis undula (Schrank, 1788).
Unidentified fragment.

TREMATODES.

Brachylaemus sp. (Dujardin, 1843).
Notocotylus attenuatus (Rudolphi, 1809).

The summarised results of the examination of adults and chickens

*According to Madsen (1949) *Heterakis gallinae* (Gmelin, 1790)=*H. gallinarum* (Schrank, 1788) and (1951) *C. collaris* is a synonym of *C. anatis* (Schrank, 1790). According to Read (1949) *C. obsignata* Madsen, 1945 is the correct name for *C. dujardini*, for the current *C. columbae*.

are shown respectively in Tables I and II. The species are listed in the tables and discussed in the notes which follow in order of their incidence within the groups Nematoda, Cestoda and Trematoda.

Heterakis gallinae.—This species has a world-wide distribution and occurs in the caeca of many species of wild and domesticated birds.

TABLE I.

The helminth parasites found in 267 adult domestic fowls.

Species of parasite	*No. of birds infested (%)*	*Total No. of worms recovered*	*Range of Nos. per infested bird*	*Mean No. per infested bird*
Heterakis gallinae ..	88.8	41,083	1–2,431	173.3
Capillaria caudinflata ..	41.2	5,585	1– 543	50.8
Capillaria collaris ..	31.1	708	1– 73	8.5
Ascaridia galli	27.0	343	1– 64	4.8
Trichostrongylus tenuis ..	3.0	37	1– 20	4.6
Capillaria dujardini ..	2.6	183	1– 97	26.1
Davainea proglottina ..	21.4	*9,660	10–1,000	169.5
Amoebotaenia sphenoides	3.4	*860	10– 200	95.6
Raillietina cesticillus ..	0.4	3	—	3.0
Cestode sp.	0.4	1	—	1.0
Notocotylus attenuatus ..	0.4	2	—	2.0
Brachylaemus sp. ..	0.4	2	—	2.0

*The numbers of individuals in infestations with this species were estimated.

A host-list compiled by Clapham (1933) included 20 genera and 33 species. British records are numerous and include those of Clapham (1938b) who found numbers up to 4,000 in birds suffering from leukaemia, and Morgan and Wilson (1938, 1939) who reported that over 80% of casualty birds examined by them were infested with this species.

During the present survey *H. gallinae* was found in 237 adult fowls (88.8%) and the average number per infested bird was 173.3. Only 6 out of the 243 birds which were parasitised by one or more species of helminths did not contain *H. gallinae*. The highest number of worms of this species found in one bird was 3,261. These were all larval forms in a chicken which also contained 527 *Capillaria caudinflata* and 41 *C. collaris*. The death of this bird was presumably due to this very heavy worm burden. The highest number of *H. gallinae* found

Table II.

The helminth parasites found in 26 chickens aged 2–12 weeks.

Species of parasite	*No. of birds infested*	*Total No. of worms recovered*	*Range of Nos. per infested bird*	*Mean No. per infested bird*
Heterakis gallinae ..	12	3,874	1–3,261	322.8
Capillaria caudinflata ..	9	997	14– 527	110.8
Capillaria collaris ..	4	71	4– 41	17.8
Capillaria dujardini ..	3	81	1– 48	27.0
Ascaridia galli	2	17	2– 15	8.5
Trichostrongylus tenuis ..	1	8	—	8.0
Davainea proglottina ..	5	*1,920	10–1,000	384.0
Dilepis undula	2	4	1– 3	2.0
Brachylaemus sp. ..	1	26	—	26.0

*The numbers of individuals in infestations with this species were estimated.

in an adult bird was 2,431, all of which were adults ; this bird, which had died from a disease of the avian leukosis complex, was additionally infested with 445 *Capillaria caudinflata*, 73 *C. collaris* and an estimated total of 200 *Davainea proglottina*. Out of the 237 infested adult fowls, 77 (32.5%) carried between 101 and 500 worms, while 20 (8.4%) carried more than 500. Two of the 12 infested chickens carried more than 100 worms.

H. gallinae normally occurred in the caeca of the host, but on a few occasions small numbers were found in the small intestine near the caecal junction, and in the rectum. The sex ratio calculated from 23,307 worms was 1 male to 1.17 females.

Capillaria caudinflata.—This species has been recorded from many gallinaceous birds in various parts of the world. Hosts include the domestic fowl (Morgan, 1932 ; Morehouse, 1944 ; and many others), the domestic turkey (Barile, 1912) and the pheasant and partridge (Clapham, 1935, 1936).

British records of *C. caudinflata* from the domestic fowl include those of Morgan and Wilson (1938) who found the species in 36.7% of the birds examined, and of Clapham (1938b) who found numbers ranging from 27 to 500 in birds suffering from leukaemia.

During the present survey *C. caudinflata* was found in 110 (42.2%) of the adult fowls examined. The average number of worms per infested bird was 50.8, and the largest number present in one bird was 548, which occurred in a bird which had died from a disease of the avian leukosis complex and which was parasitised in addition by 1,681 *Heterakis gallinae*, 2 *Capillaria collaris*, 1 *Trichostrongylus tenuis* and an estimated total of 60 *Davainea proglottina*. Nine young chickens were parasitised by *Capillaria caudinflata* and the average infestation was 110.8 worms per infested bird. Fourteen adult birds and 1 chicken carried between 100 and 500 *C. caudinflata*, and 2 adults and 1 chicken carried more than 500 worms of the species.

C. caudinflata occurred in all regions of the lower digestive tract, apart from the rectum, although the largest numbers were usually found in the duodenal loop. The sex ratio as determined from 6,590 worms was 1 male to 2.302 females.

Capillaria collaris.—This species, like those previously discussed, has a world-wide distribution in gallinaceous birds (Travassos, 1915 ; Frietas and Almeida, 1935 ; Madsen, 1945 ; and others). Previous British records include those of Morgan (1932), Foggie (1933), and Morgan and Wilson (1938).

C. collaris was found in adult fowls on 82 occasions and in young chickens on 4 occasions. Although fairly common, the parasite was never found in very large numbers, and the largest total in one bird was 73. These occurred in a fowl which had died from a disease of the avian leukosis complex, and which was parasitised additionally by 2,431 *Heterakis gallinae*, 435 *Capillaria caudinflata* and 200 *Davainea proglottina*. The mean numbers of *C. collaris* found in infested adult

birds and chickens were 8.6 and 17.8 respectively. It was always restricted to the caeca of the host, and the sex ratio as determined from 799 worms was 1 male to 1.681 females.

Ascaridia galli.—This species has been recorded from the small intestine of the domestic fowl, turkey, goose and various wild birds in most parts of the world. The occurrence of this species in Britain has been reported by, among others, Clapham (1938a) and Morgan and Wilson (1938).

During the present investigation *A. galli* was found in 72 adult fowls and 2 chickens in numbers ranging from 1 to 64. The average number of worms per infested adult was 4.8. The heaviest infestation with *A. galli* was 64 worms, which occurred in the intestine of an apparently thriving one-year-old cockerel sold for the table. The bird was infested in addition with 991 *Heterakis gallinae*, 126 *Capillaria caudinflata*, 12 *C. collaris*, 200 *Davainea proglottina* and 10 *Amoebotaenia sphenoides*.

A. galli occurred in all regions of the intestine except the rectum, although the greatest concentration was usually about midway between the duodenum and the caeca. The sex ratio calculated from 257 worms was 1 male to 1.366 females.

Capillaria dujardini.—Madsen (1945), who has summarised the literature dealing with this and many other species of Capillarid worms, stated that *C. dujardini* occurred in the domestic fowl, partridge and pigeon in many parts of the world. The species has been recorded from the domestic fowl in Britain by Foggie (1933) and Morgan and Wilson (1938).

Seven adult fowls and 3 chickens were found to be parasitised by this species during the present investigation and the average numbers were 26.1 per adult fowl and 27 per chicken. The highest number found in one bird was 97 ; these occurred in a fowl which carried in addition 308 *Heterakis gallinae*, 22 *Capillaria caudinflata* and 20 *Trichostrongylus tenuis*.

C. dujardini occurred in greatest numbers in the duodenum and first half of the ileum-jejunum of parasitised birds. The sex ratio determined from 241 worms was 1 male to 4.605 females.

Trichostrongylus tenuis.—Wehr (1945) has recorded the occurrence of *T. tenuis* in many species of domestic and wild birds in Europe and America. In Britain, Morgan and Wilson (1938) reported that the species was common in the partridge and red grouse in Scotland but relatively rare in domestic birds. Clapham (1938b) found *T. tenuis* in fowls which had died of leukaemia but not in apparently healthy

birds. Clapham (1937) had earlier failed in her attempts to infest experimental birds with this species.

During the present investigation *T. tenuis* was found in 8 adult fowls in numbers ranging from 1 to 20 with an average of 4.6 per infested bird. Five of these birds were casualty birds received by the Veterinary Investigation Officer for *post-mortem* examination, while 3 were apparently in good health. One chicken which had died from pullorum disease was infested with 8 worms.

The 45 specimens collected all occurred in the caeca of the infested birds, and the sex ratio was 1 male to 1.647 females.

Davainea proglottina.—This species is a common parasite of domestic fowls in most parts of the world and had been recorded in Britain by Meggitt (1916), Taylor (1933), Clapham (1937) and Morgan and Wilson (1938).

D. proglottina was found during the present investigation in 57 adult fowls and 5 chickens. As previously explained, the number of worms in each bird was estimated by a modified Taylor (1933) method. The main difference between the procedure carried out by Taylor and that used by the writer was that gravid proglottides were counted in the present instance, and not scolices, as it was found that many scolices remained embedded in the intestine wall where they were very difficult to see and to count. Gravid proglottides, on the other hand, could be identified with ease because of their characteristic shape and size. Although this method probably resulted in underestimation of the numbers of worms present, the observation by Levine (1938) that under optimum conditions *D. proglottina* can shed one gravid proglottis per worm per day suggests that the error was not disproportionately great. Using the above method it was found that the number of individuals per bird ranged from 10 to 1,000. The average number per infested adult was 170, and per infested chicken 380. Worms of this species were always found in the duodenal loop of infested birds, although on a few occasions when heavy infestations were present the worms extended into the first quarter of the ileum-jejunum.

Amoebotaenia sphenoides.—This species has been recovered from the small intestine of the domestic fowl in most parts of the world (Mönnig, 1947). British records include those of Meggitt (1916), Taylor (1933) and Morgan and Wilson (1938).

During the present investigation, *A. sphenoides* was found in 9 adult birds but not in any of the 26 chickens examined. Worms of this species, like *Davainea proglottina*, rapidly break up into separate

proglottides or groups of proglottides on the death of the host, and therefore the number of worms present in each infested bird was estimated as in the case of *D. proglottina*. The numbers of *A. sphenoides* per bird ranged from 10 to 200 and the average per infested bird was 95.6.

Infestations with this species were always located in the duodenal loops of the infested birds.

Other Species.—*Raillietina cesticillus*, previously recorded from domestic fowls in Britain by, among others, Meggitt (1916), and Morgan and Wilson (1938), was recovered during the present survey from only 1 bird, an adult, which was parasitised by 3 small specimens located in the small intestine.

Dilepis undula was found in the small intestines of 2 chickens, 1 of which was parasitised by 3 worms and the other by 1. Although this species is a common parasite of the blackbird (*Turdus merula merula* L.), thrush (*Turdus ericetorum ericetorum* Turton), starling (*Sturnus vulgaris vulgaris* L.) and other wild birds, there does not appear to be any previous record of its occurrence in the domestic fowl.

Two specimens of the genus *Brachylaemus* were found in the small intestine of an adult fowl, and 26 worms in the small intestine of a chicken. All the worms were immature and it was not possible to make a specific diagnosis.

Two specimens of *Notocotylus attenuatus* were found in the left caecum of an adult fowl. Dawes (1946) stated that this species was widely distributed in Europe and Asia, the hosts including the domestic fowl. *N. attenuatus* has been found in the domestic duck in Britain by Nicoll (1923) and Foggie (1933).

THE RELATIONSHIP BETWEEN THE NUMBER OF HELMINTH PARASITES AND THE NUMBER OF PARASITE SPECIES IN INFESTED ADULT FOWLS.

Although, as previously stated, the average infestation of adult fowls was 240.6 worms of 2.42 species, the numbers of worms and species present in individual fowls showed considerable variation. The number of worms per bird ranged from 1 to 1,139 and of species from 1 to 7.

When the infestations of individual birds were grouped according to the number of species comprising the infestation (Table III), it was found that the average number of worms per bird increased sharply as the number of species per bird rose from 1 to 7. Thus in birds infested with one species of parasite the average number of worms

was 51.9, while birds infested with 5 species carried on the average 736.9 worms.

On inspection, the number of worms per bird did not appear to increase in any regular manner with the number of species per bird, and in an attempt to discover a relationship between the two sets of figures, linear and quadratic equations were calculated from the data.

The equations are :—

$$Y = 157.9x - 128.2 \text{ and } Y = 32.4x^2 - 13.1x + 19.6$$

where Y = the average number of worms per bird, and x = the number of species per bird.

TABLE III.

The infestations of 267 adult fowls arranged according to the number of species of helminth parasites present.

No. of species per bird	*No. of birds*	*Mean No. of worms per bird*	*Range of Nos. per bird*
0	24	—	—
1	86	51.9	1– 437
2	52	109.2	4– 492
3	54	259.5	18– 862
4	28	560.0	34–3,139
5	18	736.9	211–1,371
6	4	819.5	261–1,341
7	1	1,884.0	—

These equations are depicted graphically in Figure 1, which includes points representing the observed data. While the linear equation provides a good indication of the rate of increase of the number of worms in relation to the number of species, an even closer fit with the observed data is shown by the quadratic equation, at least as far as the 5 species level of infestation.

Todd (1946, 1947) found that the average number of worms per bird, in birds grouped according to the number of species comprising the infestation, bore a direct relationship to the number of species

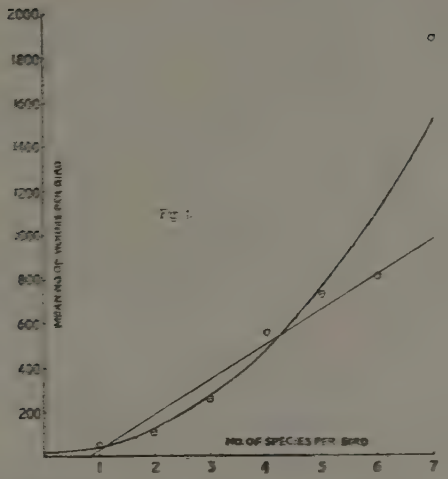

Fig. 1. The relationship between the numbers of helminth species and of worms in 267 adult fowls from Mid Wales. Graphs of the linear and quadratic equation calculated from the observed data are included.

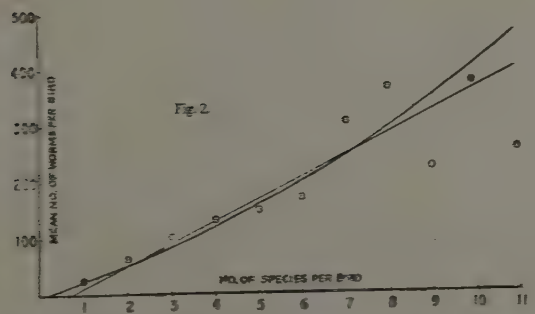

Fig. 2. The relationship between the numbers of helminth species and of worms in 753 adult fowls from Tennessee, U.S.A. Graphs of the linear and quadratic equations calculated from the observed data are included. (Data from Todd, 1946, 1947.)

present. Birds parasitised by 2, 3, 4, 5 and 6 species carried respectively 2, 3, 4, 5 and 6 times as many worms as birds parasitised by only 1 species. This relationship broke down in birds parasitised by 7 or more species, presumably because of the small numbers of birds included in these groups. The linear and quadratic equations have been calculated by the writer from Todd's data and are :—

$$Y = 29.0x - 26.5 \text{ and } Y = 1.7x^2 + 24.0x - 2.5.$$

TABLE IV.

The numbers of worms of each species found in fowls infested with from 1 to 7 species.

Species of parasite	*Mean numbers of worms of each species in birds infested with 1–7 species*						
	1	2	3	4	5	6	7
Heterakis gallinae	49.5 *(84)	97.4 (49)	200.1 (54)	437.7 (27)	391.4 (18)	347.8 (4)	1,101 (1)
Capillaria caudinflata ..	233 (1)	9.4 (22)	35.1 (39)	60.7 (26)	81.6 (17)	74.5 (4)	512 (1)
Capillaria collaris	—	5.9 (9)	7.3 (32)	9.1 (20)	12.1 (17)	8.0 (4)	3 (1)
Ascaridia galli	—	1.6 (11)	3.7 (21)	3.9 (21)	5.9 (15)	24.0 (3)	6 (1)
Davainea proglottina	60 (1)	58 (10)	107.1 (14)	170 (11)	261.3 (16)	317.5 (4)	200 (1)
Amoebotaenia sphenoides ..	—	130 (2)	10 (1)	106.7 (3)	105 (2)	60 (1)	60 (1)
Trichostrongylus tenuis ..	—	5 (1)	2 (1)	9 (3)	1 (2)	1 (1)	—
Capillaria dujardini	—	11 (1)	13 (1)	31 (4)	35 (1)	—	—

*The figures in parenthesis denote the number of birds infested in each instance.

These equations are depicted graphically in Figure 2 and it is evident that both provide a close fit with the observed data included in the figure.

While it is difficult to assess the significance of the equations derived from the data of Todd and of the writer because of the widely varying

degrees of pathogenicity and infestation-levels of the parasite species concerned, it is considered that a definite relationship has been demonstrated to exist between the numbers of parasite species and the numbers of individual parasites comprising the helminth infestations of domestic fowls.

Since the total number of worms comprising an infestation tends to increase with increase in the number of helminth species present, it is to be expected that the number of worms belonging to each individual species would also tend to increase. Morgan and Wilson (1939) observed that *Heterakis gallinae* occurred in larger numbers when heavy infestations with other species were present than when this species occurred alone or accompanied by only light infestations with other species.

The average numbers of worms belonging to 8 different species found in fowls infested by from 1 to 7 species during the present investigation are shown in Table IV. A marked tendency is evident for the average number of worms of *each* of the 5 main species to increase as the number of species per bird increases from 1 to 5. The figures per bird with 6 and 7 species of parasites do not always maintain this trend but, since the numbers of birds in the two groups were very small, the limits of the infestations with these numbers of species were not ascertained. Similarly, the incidence of the last three species in Table IV was too low to provide evidence of any tendency for the number of individuals to increase with increase in the number of species.

COMPARISON OF THE INFESTATIONS FOUND IN FOWLS OF DIFFERENT AGES.

Data on the age and sex of fowls examined for helminth parasites were obtained from sources A and B. Each bird could be referred to one of two well-defined groups, namely: cockerels culled from the main flock when between 3 and 6 months old, or hens sold for the table when between 18 and 36 months old.

Although the general levels of infestation of fowls from these sources were markedly dissimilar, birds of the younger age-group from both sources were respectively less heavily parasitised than the older birds. The infestations of birds from each of the two age-groups from sources A and B are shown in Table V. Seventeen young birds and 1 old bird from source A were free from helminth infestation, while only 2 birds from source B, both from the younger age-group, were not parasitised.

TABLE V.

The infestations of fowls aged 3–6 months compared with those of fowls over 6 months old.

Species of parasite	*Source A*				*Source B*			
	63 *fowls aged 3–6 months*		28 *fowls aged 18–36 months*		32 *fowls aged 3–6 months*		34 *fowls aged 18–36 months*	
	No. of birds infested	*Mean No. of worms per infested bird*	*No. of birds infested*	*Mean No. of worms per infested bird*	*No. of birds infested*	*Mean No. of worms per infested bird*	*No. of birds infested*	*Mean No. of worms per infested bird*
Heterakis gallinae	46 (73.0%)	58.0	27 (96.4%)	152.4	30 (93.5%)	121.7	34 (100.0%)	253.1
Capillaria caudinflata	8 (12.7%)	4.6	9 (32.1%)	21.9	17 (54.8%)	15.7	19 (55.0%)	69.7
Ascaridia galli	5 (7.9%)	1.4	14 (50.0%)	6.9	11 (35.5%)	5.6	22 (64.7%)	4.7
Capillaria collaris	— —	—	8 (28.6%)	3.6	8 (25.8%)	3.5	16 (47.1%)	11.4
Trichostrongylus tenuis	— —	—	— —	—	— —	—	1 (2.9%)	1.0
Davainea proglottina	1 (1.6%)	60.0	8 (28.6%)	63.7	10 (32.3%)	132.0	17 (50.0%)	175.3
Amoebotaenia sphenoides ..	— —	—	— —	—	— —	—	— —	—
Raillietina cesticillus	— —	—	1 (3.6%)	10.0	— —	—	1 (2.9%)	3.0

TABLE VI.

The incidence of species of parasitic helminths and the average number of worms of each species present in healthy birds from Sources A, B, and C, and in casualty birds from Source D.

Species of parasite	Source A		Source B		Source C		Source D	
	No. of birds infested %	Mean No. of worms per infested bird	No. of birds infested %	Mean No. of worms per infested bird	No. of birds infested %	Mean No. of worms per infested bird	No. of birds infested %	Mean No. of worms per infested bird
Heterakis gallinae	80.2	92.9	95.5	208.5	92.9	147.9	91.2	284.4
Ascaridia galli	20.9	5.5	50.0	5.0	19.0	4.5	17.9	3.0
Capillaria caudinflata	18.7	13.8	54.5	44.2	38.1	33.9	60.3	78.4
Capillaria collaris	9.9	3.6	36.4	8.8	38.1	10.0	48.5	9.2
Capillaria dujardini	—	—	—	—	2.5	23.0	8.8	26.7
Trichostrongylus tenuis	—	—	1.5	1.0	5.0	3.5	7.6	5.8
Davainea proglottina	9.9	63.3	40.9	159.3	16.7	284.3	26.5	155.6
Amoebotaenia sphenoides ..	1.4	10.0	—	—	7.5	153.3	7.6	78.0
Raillietina cesticillus	—	—	1.5	3.0	—	—	—	—
Cestode sp.	—	—	1.5	1.0	—	—	—	—
Notocotylus attenuatus	—	—	—	—	—	—	1.5	2.0
Brachylaemus sp.	—	—	—	—	—	—	1.5	2.0

With the sole exception of *Ascaridia galli* infestations in source B birds, each species of helminth was more widely distributed and was represented by a greater number of individuals in birds of the older age-group than in the younger birds. This difference is even more evident when the infestations are compared on the basis of the mean number of species and individual parasites per infested bird. Thus in source A, the average infested young bird carried 60.2 parasites of 1.3 species, while the average infested old bird carried 183.7 parasites of 2.5 species. The figures for source B birds, younger birds first, are 167.9 parasites of 2.4 species and 417.7 parasites of 3.3 species.

Todd (1946), working in the U.S.A., compared the infestations of birds between 8 and 26 weeks old with those of birds over 26 weeks old and found that the older birds were parasitised on the average by more than twice as many species as the younger birds and carried more than twice as many individual parasites. The data obtained in the investigations of Todd and of the writer therefore appear to support the conclusion that little or no lasting immunity towards helminth parasites is developed in adult fowls as a result of their having been infested at an early age.

THE INFESTATIONS FOUND IN FOWLS OBTAINED FROM DIFFERENT SOURCES.

As described earlier, intestines of fowls for examination were received from four different sources. Source A was an exceptionally well-managed establishment where the majority of the birds were confined in fold units which were moved, at approximately weekly intervals, on to fresh uncontaminated ground. Source B was also a well-managed poultry farm, but here the birds were kept in permanent pens; because of the high stocking-rate the pens were seldom unoccupied and the ground rarely rested. Source C included 23 farms where poultry were kept in small numbers and received no special attention; the birds were usually allowed to forage at will over land adjacent to the farm buildings. Carcases obtained from source D were those of diseased and casualty birds which had been despatched for *post-mortem* examination from many poultry-keeping establishments in Mid Wales.

The numbers of fowl intestines received for examination from sources A, B, C and D respectively were 91, 66, 42 and 68. The condensed data on the infestations of birds from each source are shown in Tables VI and VII.

Because of the relatively small number of birds examined, the absence of detailed information on the method of flock management at each of the establishments from which intestines were obtained, and the inclusion of birds of various ages and breeds, any comparison of the infestations of birds from different sources must be made with extreme caution. It is suggested, however, that the following few points are worthy of note.

Fowls maintained under the very best conditions (source A) carried infestations which were markedly lighter than those of birds from the other sources. Infested birds from source A carried an average of 105.9 parasites each (Table VII), while birds from the other three sources carried between 2 and 3 times this number. The mean

Table VII.

The average infestations present in healthy birds obtained from Sources A, B and C, and in casualty birds from Source D.

Source	*Mean No. of worms per infested bird*	*Mean No. of species per infested bird*	*% No. of birds infested with more than 100 worms*
A	105.9	1.75	20.9
B	298.6	2.86	59.1
C	224.7	2.45	45.2
D	343.7	2.83	60.3

number of parasite species per infested bird and the number of birds infested with more than 100 individual parasites were much lower in source A birds than in birds from the other sources. The data in Table VI also demonstrate the relatively light infestations found in source A birds.

It is less easy to differentiate between the levels of infestation of fowls from sources B, C and D, although source C fowls appeared to be less heavily infested than those from the other two sources. The similarity of the infestations found in birds from sources B and D, especially as shown in Table VII, is interesting in view of the fact that source D birds were casualty and diseased birds while source B birds were all apparently in healthy and thriving condition. The similarity of the infestations of birds from sources B and D suggests that the

occurrence of diseases other than helminthiasis among fowls does not necessarily result in a lowering of the birds' resistance towards invasion by helminth parasites and subsequent increase in the worm burden.

Out of the 12 species of parasites found in adult fowls, only 5 species occurred in birds from all four sources of supply, namely : *Heterakis gallinae*, *Ascaridia galli*, *Capillaria caudinflata*, *C. collaris* and *Davainea proglottina*. *Heterakis gallinae* was almost ubiquitous and the variations in the mean numbers of parasites per bird for each source could largely be accounted for by the variations in the numbers of worms of this species present.

Discussion.

The majority of examinations of poultry for helminth parasites were carried out only on the small intestine, caeca and rectum ; little information was therefore obtained on parasites such as *Syngamus trachea* and *Capillaria annulata*, which normally occur in other regions of the body. Neither of the species named was, however, found during the complete examination of 32 carcases, and it is believed that these species are not widely distributed in adult fowls in Mid Wales.

Although 12 species of parasites were found in adult fowls, only 5 were of common occurrence and were recorded in more than 20% of the birds. Control measures should therefore be directed primarily against these 5 species, and, since it was shown that the total number of parasites present in individual fowls varied with the number of species comprising the infestation, such measures should aim at reducing the number of species available to infest the birds. The average infestations of birds parasitised by one or two species respectively were 51.9 and 109.2 worms, and the largest number of worms in a single bird from either of these groups was 492 (Table III). When the number of species per bird rose to 3, the average number of worms increased to 259.5 and the peak infestation to 862. These figures, together with the rest of Table III, suggest that the possibility of fowls becoming heavily infested could be substantially reduced by preventing the number of helminth species available to infest the birds from rising above two.

The reduction of the number of species present in fowls could possibly be accomplished by dosing with anthelmintics. For example, phenothiazine has been shown by Harwood and Stunz (1945) to be effective against *Heterakis gallinae* and *Ascaridia galli*. Measures would also have to be taken to prevent the ingestion by the birds of

embryonated eggs of these species already present on the ground. Another possible method for controlling the number of helminth species is the prevention of the birds from gaining access to organisms such as slugs, earthworms and flies which act as intermediate hosts for many species of helminth parasites. The method of flock husbandry practised at source A denied, to a large extent, the access of the birds to possible intermediate hosts; the general level of infestation of birds from this source was much lower than that of fowls from any other source (Tables VI and VII). It should also be noted that the incidence of each parasite species requiring an intermediate host was markedly lower in source A birds than in the others.

Table VIII.

The helminth parasites found in 20 domestic turkeys.

Species of parasite	*No. of birds infested*	*Total No. of worms recovered*	*Range of Nos. per infested bird*	*Mean No. per infested bird*
Heterakis gallinae ..	12	700	1–385	58.3
Capillaria caudinflata ..	8	249	1–125	31.1
Capillaria collaris ..	2	11	3– 8	5.5
Ascaridia galli	1	1	—	1.0
Trichostrongylus tenuis ..	5	217	4–120	43.4
Davainea proglottina ..	1	*200	—	200.0
Amoebotaenia sphenoides	1	*200	—	200.0

*The numbers of individuals in infestations with this species were estimated.

The data provided in Table V show that older birds are generally more heavily infested than younger birds from the same flock. Any programme for the control of helminth parasites should therefore include special measures for the treatment and segregation of the older birds which otherwise apparently act as reservoir hosts and are responsible for the continual re-infestation of growing birds.

The Helminth Parasites of the Domestic Turkey (*Meleagris gallopavo dom.* (L.)).

The intestines of 20 domestic turkeys obtained in Cardiganshire, and including 11 which had died of enterohepatitis, were examined, and a summary of the infestations found is shown in Table VIII.

Five birds were free from helminth infestation, and the average infestation of the remainder was 105.2 worms of 2.0 species. The largest number of species found in a single bird was 5, when the species were *Heterakis gallinae*, *Capillaria caudinflata*, *C. collaris*, *Trichostrongylus tenuis* and *Davainea proglottina*. The most heavily infested bird carried 593 worms, and the infestation in this case comprised 385 *Heterakis gallinae*, 8 *Capillaria caudinflata* and 200 *Davainea proglottina*.

It is of interest to note that only 4 out of the 11 birds which had died of enterohepatitis were infested with *Heterakis gallinae* and that the highest number of worms of this species in any of these 4 birds was only 22. The researches of Glaser (1921), Tyzzer (1926) and others have incriminated the eggs of *H. gallinae* as the transmitting agents of *Histomonas meleagridis*, the causal agent of blackhead, but Tyzzer and Fabyan (1922) found evidence that *Heterakis gallinae* were destroyed in the caeca of birds affected by the disease.

The Helminth Parasites of the Domestic Goose (*Anser anser dom.* (L.)).

The only previous accounts of the parasites of the domestic goose in Britain appear to be those of Walton (1924) and Lewis (1930).

During the present investigation the intestines of 27 geese between 6 and 9 months old and 3 goslings, all obtained in Cardiganshire, were examined. Summaries of the infestations are shown in Tables IX and X.

Five geese were free from helminth parasites but all 3 goslings were infested. All the species found were nematodes, of which *Capillaria anseris* Madsen, 1945 was by far the most common. This species always occurred in the small intestine of the host, and normally about half-way along its length. The numbers of *C. anseris* recovered varied from 1 to 672. The closely related species *C. anatis* (Schrank, 1790) occurred in only 1 goose and 1 gosling and in each case the worms were found in the caeca of the host.

The incidence of *Amidostomum anseris* (Zeder, 1800) was probably higher than was recorded, since in the majority of cases gizzards were not obtained for examination.

The Helminth Parasites of the Domestic Duck (*Anas platyrhyncha dom.* (L.)).

Previous accounts of the helminth parasites of domestic ducks in Britain include those of Lewis (1930), Foggie (1933) and Davies (1938),

TABLE IX.

The helminth parasites found in 27 adult domestic geese.

Species of parasite	*No. of birds infested*	*Total No. of worms recovered*	*Range of Nos. per infested bird*	*Mean No. per infested bird*
Capillaria anseris ..	17	1,033	1–672	60.8
**Amidostomum anseris* ..	7	—	—	—
Trichostrongylus tenuis ..	5	31	2– 17	6.2
Heterakis gallinae ..	3	7	1– 4	2.3
Capillaria anatis ..	1	1	—	1.0

*Counts of the numbers of this species were not made as in most cases the gizzard was not received for examination.

TABLE X.

The helminth parasites found in 3 goslings.

Species of parasite	*No. of birds infested*	*Total No. of worms recovered*	*Range of Nos. per infested bird*	*Mean No. per infested bird*
Capillaria anseris ..	3	150	15– 20	50.0
**Amidostomum anseris* ..	3	—	—	—
Trichostrongylus tenuis ..	3	98	14– 65	32.7
Heterakis gallinae ..	3	175	4–156	58.3
Capillaria caudinflata ..	1	56	—	56.0
Capillaria anatis ..	1	15	—	15.0

*Counts of the numbers of this species were not made as in most cases the gizzard was not received for examination.

who between them recorded the occurrence in ducks of 5 species of trematodes, 9 cestodes, 3 nematodes and 1 acanthocephalid.

During the present investigation the intestines of 47 ducks between the ages of 5 and 10 months were examined. Nineteen were free from infestation and the remainder were relatively lightly parasitised by one or more of the following 10 species :—

NEMATODES.

Capillaria anatis (Schrank, 1790).
Tetrameres fissispina (Diesing, 1861).

CESTODES.

Aploparaksis furcigera (Rudolphi, 1819).
Hymenolepis gracilis (Zeder, 1803).
Hymenolepis coronula (Dujardin, 1845).
Hymenolepis sp.
Fimbriaria fasciolaris (Pallas, 1781).
Unidentified fragment.

TREMATODES.

Echinoparyphium recurvatum (v. Linstow, 1873) Lühe, 1909.
Hypoderaeum conoideum (Bloch, 1782) Dietz, 1909.

The summarised data on the infestations are shown in Table XI.

The most important record is probably that of *Tetrameres fissispina*, as it is known that this species can cause serious losses among ducks (Lange, 1938). Although common in certain parts of the world, *T. fissispina* has not frequently been found in British birds. Foggie (1933) found 4 males in the proventriculus of one duck, and Watkins (1947) reported a more recent occurrence of this species in ducks from a farm in Southern England. Baylis (1928) found specimens in a scaup-duck (*Nyroca marila marila* (L.)) and a common scoter (*Melanitta nigra nigra* (L.)).

In addition to the occurrence of *T. fissispina* in 3 ducks, this species was found on 4 occasions during the examination of a number of wild birds received at the laboratory. The infested birds were, woodcock (*Scolopax rusticola rusticola* (L.)), common snipe (*Capella gallinago gallinago* (L.)), scaup-duck (*Nyroca marila marila* (L.)) and tawny owl (*Strix aluco sylvatica* Shaw).

The Helminth Parasites of the Muscovy Duck (*Cairina moschata* (L.)).

The intestines of 10 muscovy ducks from a farm near Aberystwyth were obtained for examination. Five of the birds were not infested

with helminth parasites. The remaining five birds were parasitised by *Capillaria anatis*, which occurred in the caeca in numbers of 1, 4, 34, 35 and 63 respectively. The last bird, which died at the age of 6 weeks from an unknown cause, was parasitised additionally by 647 *C. caudinflata* and 13 *Ascaridia galli*.

Table XI.

The helminth parasites found in 47 domestic ducks.

Species of parasite	*No. of birds infested*	*Total No. of worms recovered*	*Range of Nos. per infested bird*	*Mean No. per infested bird*
Capillaria anatis ..	9	17	1– 4	1.9
Tetrameres fissipina ..	3	34	7–19	11.3
Aploparaksis furcigera ..	12	34	1– 9	2.8
Hymenolepis gracilis ..	7	50	1–25	7.1
Hymenolepis coronula ..	4	22	2–11	5.5
Hymenolepis sp. ..	1	4	—	4.0
Fimbriaria fasciolaris ..	1	1	—	1.0
Cestode sp. (Unidentified)	1	1	—	1.0
Echinoparyphium recurvatum	2	45	3–42	22.5
Hypoderaeum conoideum	1	1	—	1.0

Summary.

1. The lower intestinal tracts (duodenum to rectum) of 267 adult domestic fowls were examined for helminth parasites and 91% were found to be infested. The average numbers of worms and species present in infested birds were 240.6 and 2.42 respectively. Twelve species of parasites were recovered but only 5 occurred in more than 20% of the birds.

2. Twenty-six casualty and diseased chickens between 2 and 12 weeks old were examined. Fourteen were infested with helminth parasites and the average infestation was 499.9 worms of 2.79 species.

3. The total numbers of worms of all species present in individual fowls were shown to bear a relationship to the number of species comprising the infestations. Linear and quadratic equations which demonstrate the relationship have been derived from the observed figures.

4. Fowls over 18 months old were infested by more than double the number of worms carried by birds between 3 and 6 months old.

5. Fowls maintained under the very best conditions of management carried infestations markedly lower than those carried by healthy birds under more normal conditions, and by casualty and diseased birds.

6. The implications of the results obtained are discussed briefly and measures for the control of helminth infestation suggested.

7. The results of the examination of small numbers of turkeys, geese, ducks and muscovy ducks are presented.

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*Experimental Studies on Egg Development, Hatching and Retrofection in *Aspiculuris tetraptera*.

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Langhans (1926) stated that the larvae of *Enterobius vermicularis* were found in the anal region. He believed that the mature worm migrated downwards from the intestine and laid eggs in the anal opening. If the situation was very good and the temperature was suitable, the larvae might hatch out and migrate into the bowel because the temperature there was higher than that of the exterior. Hamburger (1939) supported this view, but he had no experimental evidence. Madsen (1945) on the other hand was much opposed to it and in the last 24 years nobody has reported the finding of *E. vermicularis* larvae in the anal region.

Schuffner and Swellengrebel (1949), after citing Langhans' paper, describe how they applied *E. vermicularis* larvae, hatched from artificial gastric juice, to the sphincter portion of the anus of three volunteers and they all became infected. This mode of infection, namely retrograde migration of larvae hatched in the anal region, was designated " retrofection."

Recently, Prince (1950) found larvae of *Syphacia obvelata* in the anal region of infected rats. He stated that " a rat was lightly anaesthetized to prevent struggling, and its anal region was washed with 10% alcohol. The washing was caught in a glass container. This liquid was then centrifuged and the sediment examined. A fairly large number of embryonated eggs and some larvae measuring 0.14 to 0.22 mm. in length were obtained in this way. In addition to unhatched embryonated eggs found on the anal region, about 25% of the eggs found were actually empty shells. Using water in place of the 10% alcohol, living larvae as small as 0.09 mm. in length have been recovered." Although his study throws some light on retrofection in rats, the evidence is still inconclusive. To investigate the

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problem further, the writer considered two methods of approach (1) direct, by applying infective eggs of *E. vermicularis* to the anal opening to follow up whether they hatched and migrated into the bowel, (2) indirect, by studying animal oxyurids to find out whether or not retrofection occurred. Since the eggs of *E. vermicularis* were very difficult to obtain, the following studies are mainly concerned with the murine oxyurid, *Aspiculuris tetraptera* (Nitzsch, 1821).

Before attempting to demonstrate the occurrence of retrofection in *A. tetraptera*, observations were made on the development and hatching of the eggs of this species and also those of *E. vermicularis* when these were available.

The Development of the Eggs of *Aspiculuris tetraptera* and *Enterobius vermicularis*.

Philpot (1924) stated that eggs contained in the uterus of the mature female of *Aspiculuris tetraptera* and those from the rectum and faeces of the host were in the segmented stage. The eggs developed very rapidly when deposited by or pressed from the mature female. Embryos moving within the shell were found after 68 hours' incubation in water at 22°C. A similar stage was reached in about 20 hours at 37°C. She found that development also proceeded very readily in saliva. In the case of *E. vermicularis*, she stated that at 37°C. in water, the eggs showed coiled moving embryos when examined after five and three-quarter hours.

Zawadowsky and Schalimov (1929) stated that the eggs of threadworms would develop further after leaving the body only if they had reached the tadpole stage. If so, they would develop in normal saline, in water, in saturated copper sulphate solution, in formalin and other media.

Heller (1944) studied the epidemiology of enterobiasis and showed that the optimal temperature for the development of the eggs was 34 to 36°C., at which temperature they reached the infective stage in 4–6 hours. The human peri-anal region is very favourable to their development and eggs fixed there in silken bags reached the infective stage in 4–5 hours.

Personal Observations.

The development of the eggs of A. tetraptera *in normal horse serum.*

To discover whether eggs in serum discharged by haemorrhoids are readily developed or not, they were cultured in serum. The eggs

were dissected out from the uterus of the adult worm and incubated in normal horse serum at 25°C. Seventeen hours later, the eggs contained small vermiform embryos. After 41 hours, the embryos had increased in length and after 65 hours' incubation they were moving in their shells.

Comparison of the development of the eggs of Aspiculuris tetraptera *in saline solutions of different concentrations.*

The principal component of sweat is sodium chloride and its concentration varies from 0.43 to 0.83 per cent. (Talbert and Haugen, 1927). To determine, therefore, whether the concentration of saline affects development, the eggs were incubated in different solutions. The eggs used for the experiment were isolated from the faeces of six infected mice which were put in a large glass cage for one night. Their faeces were collected and comminuted in a large amount of water, and allowed to settle for half an hour ; the top two-thirds of the fluid and floating débris were then poured off. The sediment was resuspended with water and allowed to settle again. The upper part of the fluid was poured off and the lower part was strained through a wire basket. The strained suspension was mixed with water, allowed to settle for half an hour and the supernatant fluid was poured off. This procedure was repeated several times, until the supernatant fluid was clear. The sediment was placed in a centrifuge tube and spun for ten minutes. The fluid was poured off. The sediment was resuspended in saturated saline and spun for another ten minutes. The eggs floating on the surface of the saline were removed by a loop and placed in a tube containing distilled water to wash out the sodium chloride. They had just developed into the segmented stage. The eggs were then transferred to watch glasses (5.3 cm. diameter) for incubation, each watch glass containing about 150 eggs. The watch glasses were sterilized in a dry heat autoclave at 140°C. for half an hour and the eggs were incubated in them in about 2 to 3 c.c. of each of the various solutions to be tested. To prevent evaporation each watch glass was kept in a Petri dish containing a few c.c. of water and this was labelled with the name and concentration of the solution, the number of the dish, the temperature and the date. The Petri dishes and their contents were incubated at 28°C. or 37°C. for various periods after which the numbers of eggs were counted and the percentage which developed was calculated.

The eggs were incubated at 37°C. for 17 hours in four different solutions, i.e., 0.85 per cent., 0.65 per cent., 0.45 per cent., 0.25 per

cent. saline and also in pure water. The percentages of developed eggs corresponding to the above solutions were 87.9, 90.4, 93.2, 88.9 and 89.1 in water. It is apparent that below 0.85 per cent., the saline concentration has no great influence on the development of the embryo.

Comparison of the development of the eggs of Aspiculuris tetraptera *in horse serum and water.*

Eggs were isolated from a mouse's faeces which had been discharged within the previous 18 hours. They were in the segmented stage. They were transferred to four watch glasses, two of them containing water, two of them containing horse serum. Two watch glasses (one containing water and one serum) were kept at 28°C. and the other two at 37°C. for 24 hours. On examination, most of the eggs were fully developed and in some the embryos were moving in their shells. The only difference observed was that the proportion of moving larvae was greater in the watch glasses kept at 28°C. (4 in 5) than in those kept at 37°C. (1 in 8).

Comparison of the development of the eggs of Enterobius vermicularis *in human serum and in water.*

Eggs were discharged on a slide by a mature female worm obtained from enema specimens. They were transferred to two watch glasses (one containing water and the other containing human serum) and kept at 37°C. for three hours. Some of the eggs were then taken out from each liquid for examination and were found to have developed to the tadpole stage. Five hours later, moving vermiform embryos were found in their shells. From this experiment it is concluded that both serum and water are suitable for the development of the eggs of *E. vermicularis*.

The Hatching of the Eggs of *Aspiculuris tetraptera* and *Enterobius vermicularis* in different Media.

In the investigation of retrofection, the factors affecting the hatching of the eggs of *A. tetraptera* and *E. vermicularis* require thorough study. Under what conditions can the eggs hatch out ? Are there optimum conditions for hatching ? To answer these questions, a series of experiments was performed by the writer. Before describing these, it is appropriate to refer to previous work on this subject.

Zenker (1872) (see Cobb, 1890) found the larvae of *Oxyuris vermicularis* in the duodenum and small intestine. Cobb (1890) hatched out the larvae of *O. vermicularis* in the human stomach under normal

conditions. He swallowed a " suction capsule " in which was placed a large number of eggs, containing fully developed larvae, which moved actively when warmed. Six hours later, he recovered it by means of purging. On opening the capsule on a warm stage, he found a large number of actively moving larvae. The capsule contained acid fluid. Gordon and Macfie (1924) hatched the eggs of *Oxyuris equi* in a solution of sodium carbonate (0.1 per cent. to 0.5 per cent.) after immersion of the eggs in hydrochloric acid (0.1 to 0.2 per cent.) for half an hour. The larvae were very active. Philpot (1924) hatched the eggs of *E. vermicularis* in water at 22°C., 25°C. and 37°C. and in saliva at 37°C. She tested the following solutions (1) 0.2 per cent. hydrochloric acid, (2) 1 per cent. trypsin and 0.5 per cent. sodium bicarbonate and (3) 0.3 per cent. pepsin and 0.1 per cent. hydrochloric acid. She found that all of these could kill the tadpole stage of the eggs, but (2) and (3) could hatch out the eggs containing coiled embryos. She also hatched the eggs of *Aspiculuris tetraptera* and stated that embryos could be freed from their shells to a certain extent by stirring the culture quickly or by allowing it to dry and then re-moistening it. The freed embryos showed no movement in water. Emergence from the shell was also induced both in 0.5 per cent. and 0.8 per cent. sodium bicarbonate.

Lentze (1932) tested the infectivity of the eggs of *E. vermicularis* by feeding the eggs to mice and later examining their intestines.

Jones and Jacobs (1941) used the hatching technique to study the survival of the eggs of *E. vermicularis* under known conditions of humidity and temperature. They hatched the eggs in a solution of 0.4 c.c. of distilled water and 0.6 c.c. of artificial gastric juice (0.7 per cent. HCl and 0.5 per cent. pepsin) at 37°C.

Personal Observations.

In the experiments to be described the eggs used were isolated from a mouse's faeces which were collected from its cage. They were hatched in a watch glass by the same technique as that used for study on the development of the eggs of *Aspiculuris tetraptera*. The technique was gradually developed by the writer. At first the eggs were hatched in a small Petri dish, but because its bottom was flat, the eggs were not easy to find. Latterly, a watch glass was used. Since fungus was found to grow in the culture, the container was sterilized. After this technique had been developed, however, it was found that Yoshida and Toyoda (1938) had used the same method to hatch *Ascaris* eggs.

1. *The hatching of* A. tetraptera *eggs in artificial gastric juice.*

Three series of experiments were carried out at different times :

(*a*) The eggs of *A. tetraptera* were put into two watch glasses containing artificial gastric juice (0.7 per cent. HCl and 1 per cent. pepsin). One was incubated at 28°C., the other at 37°C. Seventeen hours later, 6 per cent. of those eggs incubated at 28°C. had hatched out, whereas no hatching occurred in those incubated at 37°C.

TABLE I.

Results of Hatching *A. tetraptera* Eggs in Artificial Gastric Juice.

% Gastric Juice in Water	Temperature	No. of Eggs	Hours of Incubation	Percentage Hatching
50	28°C.	104	4½	FL 17.4 EM 25.4 UE 57.2
50	37°C.	130	4½	FL 29.3 EM 36 UE 34.7
25	37°C.	160	17	FL 32.5 EM 32.5 UE 35

FL = Free larvae ; EM = Emerging larvae ; UE = Unhatched eggs.

(*b*) The eggs were put into two watch glasses containing 50 per cent. artificial gastric juice in distilled water. The results are shown in Table I. It was shown that in these dilute solutions the eggs were readily hatched. The percentage of free larvae and of emerging larvae was much higher among those incubated at 37°C.

(*c*) The eggs were put into a watch glass containing 25 per cent. artificial gastric juice in distilled water and were incubated at 37°C. When they were examined 17 hours later, there were 32.5 per cent. free larvae and after 65 hours' incubation all the eggs had hatched.

It has been mentioned above that Jones and Jacobs (1941) also hatched eggs of *E. vermicularis* very successfully in dilute artificial gastric juice which shows that the eggs of these two parasites resemble one another in this respect.

2. *The hatching of* A. tetraptera *eggs in dilute hydrochloric acid.*

As it is well known that faeces are sometimes acid and sometimes alkaline, it was of interest to determine whether the eggs of Oxyurids are more readily hatched under acid or alkaline conditions. To investigate this problem, a series of experiments was carried out.

TABLE II.

Results of Hatching *A. tetraptera* Eggs in Dilute HCl.

Concentration of Solution	Temperature	No. of Eggs	Hours of Incubation	Percentage Hatching
N/50	37°C.	111	4	FL 10.8 EM 2.7 UE 86.5
N/50	28°C.	108	4	FL 5.5 EM 3.7 UE 90.8
N/25	37°C.	105	4	FL 8.5 EM 5.7 UE 85.8

FL = Free larvae ; EM = Emerging larvae ; UE = Unhatched eggs.

Hydrochloric acid solutions were prepared in two concentrations. Solution (A) consisted of N/50 hydrochloric acid and Solution (B) N/25 hydrochloric acid. Each solution, together with eggs of *A. tetraptera*, was placed in two watch glasses, one of which was incubated at 28°C. and the other at 37°C. (The watch glass which contained Solution (B) and was kept at 28°C. was broken by accident.) The result of hatching is shown in Table II. The proportions of free larvae in these solutions are rather small. About 60 per cent. of the vermiform embryos in their shells appeared to be dead. Some embryos were still moving in their shells. 29 hours later, all the free larvae in these solutions appeared to be dead.

3. *The hatching of* A. tetraptera *eggs in sodium bicarbonate solution.*

0.5 per cent. and 0.25 per cent. sodium bicarbonate solutions were used in these experiments. Two watch glasses were prepared containing each solution, one being kept at 37°C. and the other at 28°C. Five hours later, no hatching had taken place in either of these solutions. After 25 hours' incubation, both free larvae and emerging larvae were observed in the percentages shown in Table III. In the

Table III.

Results of Hatching *A. tetraptera* Eggs in Sodium Bicarbonate Solution.

Solution Percentage	Temperature	No. of Eggs	Percentage Hatching		
			Hours of Incubation		
			25	48	79
0.5	37°C.	250	FL 21.2 EM 36.5 UE 42.3	32.7 42.1 25.2	34 44 22
0.5	28°C.	160	FL 0 EM 2.5 UE 97.5	0 4.8 95.2	0 6 94
0.25	37°C.	159	FL 19.5 EM 11.3 UE 69.2	41.2 16.4 42.6	48 23 29
0.25	28°C.	150	FL 0 EM 1 UE 99	1 4 95	4 5 91

FL = Free larvae; EM = Emerging larvae; UE = Unhatched eggs.

0.5 per cent. solution, incubated at 37°C., 21.2 per cent. free larvae, 36.5 per cent. emerging larvae and 42.3 per cent. unhatched eggs were found, whereas at 28°C. there were 2.5 per cent. emerging larvae and 97.5 per cent. unhatched eggs. In the 0.25 per cent. solution, incubated at 37°C., 19.5 per cent. free larvae, 11.3 per cent. emerging larvae and 69.2 per cent. unhatched eggs were found. When incubated at 28°C. there were 1 per cent. emerging larvae and 99 per cent. unhatched eggs and there were no free larvae. It is clear that the 0.5 per cent. solution

is much more suitable for the hatching than the 0.25 per cent. solution and the temperature (37°C.) is also important. The free larvae found in the 0.5 per cent. solution had no movement and those in the 0.25 per cent. solution were moving sluggishly.

After 48 hours' incubation, in the 0.5 per cent. solution at 37°C., 25.2 per cent. of the eggs were still unhatched, whereas in the 0.25 per cent. solution, at the same temperature, the proportion was 42.6 per cent. At 28°C., both in 0.5 per cent. and 0.25 per cent. solutions, the percentage of unhatched eggs was about 95. The free larvae found in the solution appeared to be dead.

After 79 hours' incubation, in both 0.5 and 0.25 per cent. solutions at 37°C., the larvae appeared to be dead.

4. *The hatching of* A. tetraptera *eggs in normal horse serum.*

Since we conjectured that the eggs might readily hatch out in serum discharged by haemorrhoids and then migrate upwards causing an infection, we incubated the eggs of *A. tetraptera* in horse serum and followed the results. Two series of solutions were prepared. One was incubated at 37°C. and the other at 28°C. In each series, three watch glasses were prepared. The first contained 10 per cent. serum in water, the second contained 20 per cent. serum in water and the third contained whole serum.

The eggs of *A. tetraptera* used in the experiment had just been isolated from mice faeces and had not been ripened. After 19 hours' incubation, segmented embryos had developed into the vermiform embryo stages but no free larva was found in any of the solutions. The result of hatching for 42 hours is shown in Table IV. In the series incubated at 37°C., the percentage of hatching is greater in the dilute solution. In the 10 per cent. serum, two emerging larvae were moving actively. The solutions incubated at 28°C. had turned red. No hatching had occurred.

After 90 hours' incubation, all watch glasses were examined. In the 37°C. series, the percentage of hatching, both in the 10 per cent. serum and in the 20 per cent. serum, had somewhat increased. In the whole serum, 9 per cent. emerging larvae and 91 per cent. unhatched eggs but no free larvae were found. Some larvae in their shells appeared to be dead. In the 28°C. series, small percentages of free larvae were found both in 10 per cent. and in 20 per cent. serum. In the whole serum, there was no hatching after 90 hours, but after 119 hours, 9.7 per cent. emerging larvae were found.

Another experiment was carried out to decide whether we could obtain a high percentage of hatching by continuing to transfer the eggs into fresh horse serum. We used eggs of *A. tetraptera* which had been cultured at 25°C. for 3 days. 123 eggs were put into the horse serum and incubated at 37°C. 19 hours later, no free larvae nor emerging larvae were found. The eggs were then transferred to a watch glass containing fresh serum and kept at the same temperature.

TABLE IV.

Results of Hatching *A. tetraptera* Eggs in Horse Serum.

Percentage Serum	Temperature	No. of Eggs	Percentage Hatching	
			Hours of Incubation	
			42	90
10	37°C.	129	FL 28.2 EM 11.7 UE 61.2	64 8 28
20	37°C.	144	FL 8.6 EM 8.6 UE 82.8	44.1 8.9 47
100	37°C.	121	FL 0 EM 6.3 UE 93.7	0 9 91
10	28°C.	101	FL 0 EM 0 UE 100	3 0 97
20	28°C.	113	FL 0 EM 0 UE 100	2.8 0 97.2
100	28°C.	177	FL 0 EM 0 UE 100	0 0 100

FL = Free larvae ; EM = Emerging larvae ; UE = Unhatched eggs.

24 hours later (after a total of 43 hours' incubation) 1.2 per cent. emerging larvae and 98.8 per cent. unhatched eggs were found. The unhatched eggs were transferred to fresh serum again. 24 hours later (after 67 hours' incubation in all), 24 per cent. emerging larvae and 76 per cent. unhatched eggs were found.

TABLE V.

Results of Hatching *A. tetraptera* Eggs in Saline.

Percentage Saline	Temperature °	No. of Eggs	Percentage Hatching		
			Hours of Incubation		
			19	43	69
0.28	37°C.	138	FL 9.8 EM 11 UE 79.2	19.3 8 72.7	21.7 6.4 71.9
0.28	28°C.	107	FL 0 EM 6.4 UE 93.6	1 7 92	18.7 6.2 75.1

FL = Free larvae ; EM = Emerging larvae ; UE = Unhatched eggs.

5. *The hatching of* A. tetraptera *eggs in human sweat.*

If the eggs can hatch out in sweat, it is possible that they may give rise to retrofection in this way. To investigate it, 247 eggs were placed in sweat and incubated at 37°C. Three hours later, no hatching was found. Vermiform embryos were moving in their shells. After seven hours' incubation, the result was the same. After 26 hours' incubation, only 1 emerging larva was found. The sweat had a foetid smell. The vermiform embryos had no movement. After 53 hours' incubation, 92 per cent. of the larvae had degenerated. The experiment was repeated with the same result. Thus sweat is shown to be unsuitable for the hatching of *A. tetraptera* eggs.

6. *The hatching of* A. tetraptera *eggs in saline.*

The failure of *A. tetraptera* eggs to hatch in human sweat may be due to the fermentation of the sweat inhibiting the process of the hatching. The principal component of sweat is sodium chloride. Eggs were therefore incubated in dilute saline at 37°C. and 28°C. The results are shown in Table V which shows that 37°C. is more suitable

for hatching. Free larvae moving actively in the solution were found.

7. *The hatching of* A. tetraptera *eggs in albumin solution.*

Egg albumin was used. Three series of watch glasses were prepared. In (*a*) two watch glasses were filled with albumin, in (*b*) two watch glasses were filled with 10 per cent. albumin in distilled water and in (*c*) two watch glasses were filled with 5 per cent. albumin in distilled water. Eggs were transferred into each watch glass and one watch glass from each series *a*, *b* and *c* was incubated at 37°C. and the other at 28°C. The results are shown in Table VI which shows that the eggs readily hatch at 37°C. At this temperature, the percentage of hatching gradually increased with time. The 10 per cent. albumin solution was the most suitable for hatching. After 47 hours' incubation, 24.2 per cent. of the eggs in this solution had hatched, while in pure albumin only 6.4 per cent. had hatched and in 5 per cent. albumin only 14.2 per cent. After 118 hours' incubation, the percentage of hatched eggs reached its maximum of 89.2 per cent. (in 10 per cent. albumin solution). At 28°C. the eggs did not hatch for the first 4 days ; by the fifth day a few had hatched.

8. *The hatching of* A. tetraptera *eggs in the urine of mice.*

The eggs of *A. tetraptera* were isolated from mice faeces and incubated at 25°C. for 6 days. 180 eggs were then transferred to mice urine and incubated at 37°C. 24 hours later, 41.2 per cent. free larvae and 38.8 per cent. emerging larvae were found. After 48 hours' incubation, 91.9 per cent. empty shells were found. After 90 hours in all, 97 per cent. empty shells were found. Although the larvae hatched out, in no instance was any movement observed. This indicates that the larvae cannot survive for long in urine.

9. *The hatching of* A. tetraptera *eggs in water.*

A large number of *A. tetraptera* eggs was isolated from mice faeces and incubated at 28°C. in a small Petri dish. In the first three weeks, no free larva was found and the vermiform embryos in their shells had a normal appearance. One month later, free larvae and emerging larvae were found. After two months' incubation, they were still being found. The vermiform embryos in the shells appeared to be alive and most of the eggs had hatched. After a total of three months, free larvae were still found. In the shells, the embryos appeared to be normal but some were degenerate.

Eggs of *A. tetraptera* containing vermiform embryos in their shells were dried for 24 hours. They were moistened by water and incubated at 28°C. for 17 hours. On examination, 6.4 per cent. free larvae and 12.9 per cent. emerging larvae were found.

TABLE VI.

Results of Hatching *A. tetraptera* Eggs in Albumin.

Percentage Albumin	Temperature	No. of Eggs	Percentage Hatching			
			Hours of Incubation			
			22	47	70	118
100	37°C.	120	FL 0 EM 1.1 UE 98.9	3.2 3.2 93.6	10.1 8.9 81.0	78.1 6.8 15.1
10	37°C.	83	FL 1.4 EM 2.9 UE 95.7	13.9 10.3 75.8	56.4 11.6 32	89.2 0 10.8
5	37°C.	167	FL 1.2 EM .6 UE 98.2	5.4 8.8 85.8	50 17.2 32.8	85 0 14.1
100	28°C.	110	FL 0 EM 0 UE 100	0 0 100	0 0 100	7.7 0 92.3
10	28°C.	181	FL 0 EM 0 UE 100	0 0 100	0 0 100	19 0 81
5	28°C.	94	FL 0 EM 0 UE 100	0 0 100	0 0 100	13.7 0 86.3

FL = Free larvae; EM = Emerging larvae; UE = Unhatched eggs.

10. *The hatching of* A. tetraptera *eggs in sugar solution.*

0.1 per cent. aqueous sugar solution was prepared. The eggs of *A. tetraptera* with developed vermiform embryos were put into the solution. 48 hours later, a few free larvae and emerging larvae were found. The results are shown in Table VII. After 120 hours' incubation, the percentage of hatching was still small. The experiment was repeated with the same result.

TABLE VII.

Results of Hatching *A. tetraptera* Eggs in Sugar Solution.

Percentage Solution	Temperature	No. of Eggs	Percentage Hatching	
			Hours of Incubation	
			48	120
0.1	28°C.	110	FL 1 EM 0 UE 99	2.6 1.3 96.1
0.1	37°C.	100	FL 3.1 EM 14.3 UE 82.6	10 8 81.3

FL = Free larvae; EM = Emerging larvae; UE = Unhatched eggs.

11. *The hatching* of A. tetraptera *eggs in Locke's solution.*

Three series of solutions were prepared: (*a*) Locke's solution, (*b*) 50 per cent. Locke's solution in water and (*c*) 25 per cent. Locke's solution in water. Each solution was used for preparing two watch glasses. One watch glass was incubated at 37°C. and the other at 28°C. Four hours later, only one emerging larva was found in Locke's solution incubated at 37°C. After 98 hours' incubation, the number of eggs hatching at 37°C. was in inverse proportion to the concentration of the solution. In Locke's solution, the free larvae and emerging larvae were 3 per cent. In 50 per cent. Locke's solution they were 11 per cent.; in 25 per cent. Locke's solution they were 32.8 per cent. The eggs which were incubated at 28°C. had 1 per cent. emerging larvae in 25 per cent. Locke's solution. The results are shown in Table VIII.

TABLE VIII.

Results of Hatching *A. tetraptera* Eggs in Locke's Solution.

Percentage Solution	Temperature	No. of Eggs	Percentage Hatching	
			Hours of Incubation	
			4	98
100	37°C.	100	FL 0 EM 1 UE 99	1 2 97
50	37°C.	100	FL 0 EM 0 UE 100	4 7 89
25	37°C.	119	FL 0 EM 0 UE 100	22.6 10.2 67.2
100	28°C.	100	FL 0 EM 0 UE 100	0 0 100
50	28°C.	100	FL 0 EM 0 UE 100	0 0 100
25	28°C.	100	FL 0 EM 0 UE 100	0 1 99

FL = Free larvae; EM = Emerging larvae; UE = Unhatched eggs.

12. *The hatching of* A. tetraptera *eggs in dilute gastric juice and sodium bicarbonate solution.*

Eggs of *A. tetraptera* were incubated in 50 per cent. gastric juice in water for half an hour at 37°C. Then they were transferred to 0.5 per cent. sodium bicarbonate solution and incubated at 37°C. for four hours. No free larva was found. After 21 hours' incubation, free larvae were present but these were motionless.

Observations on the Bionomics of the Larvae of *Aspiculuris tetraptera.*

About 30 eggs of *A. tetraptera* were transferred to 0.25 per cent. saline and incubated at 37°C. 17 hours later, 4 free larvae were found in the solution. After 21 hours' incubation there were 7 free larvae, 4 of them moving actively. The unhatched eggs were removed and afterwards the larvae were examined regularly at two-hour intervals. One of the 7 larvae lived 44 hours while 3 of them lived 28 hours. On the first day they moved actively but on the second day they were sluggish. In two other observations, 7 larvae lived for 25 hours but were not very active.

It was observed that larvae emerging from their egg shells have active movements which later become abated. Since we thought that this phenomenon might be caused by the lower temperature during the time of examination, further observations were therefore made on a warm stage.

Larvae hatched in 0.25 per cent. saline were transferred to saline on a hollow slide and covered with a cover glass. When the slide was put on a warm stage kept at about 35°C. the larvae were seen to curve and straighten actively but they did not migrate.

Measurements were made at intervals on two larvae with the following results :

First measurement :

No. 1. Length 131.75μ ; width 23.375μ ;
Length of oesophagus 55.25μ.

No. 2. Length 136.00μ ; width 25.5μ ;
Length of oesophagus 55.25μ.

The slide was then put into a Petri dish containing a few drops of water and kept at 37°C. for 18 hours, after which they were measured again.

Second measurement :

No. 1. Length 148.75μ ; width 23.375μ ;
Length of oesophagus 55.25μ.
No. 2. Length 165.75μ ; width 25.5μ ;
Length of oesophagus 55.25μ.

Their movement at this time was sluggish. They were incubated at 37°C. for another 24 hours and again measured.

Third measurement :

No. 1. Length 170μ ; width 23.375μ.
No. 2. Length 182.75μ ; width 25.5μ.

These two larvae had died and were somewhat degenerate. The posterior ends of the oesophagus in both were not clear and this structure could not be measured.

Another two larvae were transferred to a hollow slide from 0.45 per cent. saline and kept at room temperature. These had very sluggish movements and did not increase in size even after 30 hours.

The Hatching of Eggs of *Enterobius vermicularis.*

To obtain material for this study, enema specimens from persons infected with *E. vermicularis* were brought from hospital. The faeces were comminuted and mixed in a large amount of tap water in a tray and after sedimentation the supernatant fluid was decanted. This procedure was repeated several times until the water in the tray was clear. The worms were picked out and washed in normal saline and then transferred to clean distilled water or normal saline. Eggs were obtained after being discharged by the living worms or, in a few cases, the gravid worms were transferred to a clean slide to let them discharge their eggs, which were then used for our experimental work. Most of the worms collected from enema specimens were not fully developed so that the eggs obtained from them were not very satisfactory for the hatching test because most of them were immature. Frequently the eggs did not develop at all and sometimes only a small proportion of them developed. In only a few cases did the eggs develop very well. Although this was not altogether satisfactory, it was the only way available for us to obtain material for experimental work. Gravid female worms were selected and used for the following experiments :

(1) The eggs were dissected out from a gravid female *E. vermicularis* and incubated in water at 28°C. for 18 hours. Vermiform

embryos developed in all of them and many free larvae were seen moving actively in the water. More than 100 eggs were counted, of which 11.7 per cent. were empty shells, 29.1 per cent. were in the act of hatching and 59.2 per cent. were unhatched eggs. Five hours after this counting, the free larvae were dead. After 42 hours' incubation, there were 85.5 per cent. empty shells, 1 per cent. hatching and 13.5 per cent. unhatched eggs. After a total of 66 hours' incubation, 98 per cent. empty shells and 2 per cent. unhatched eggs remained.

(2) 150 eggs discharged by a female *E. vermicularis* were incubated in water at 37°C. for 16 hours, at the end of which time 5.5 per cent. free larvae, 30.5 per cent. hatching and 64 per cent. unhatched eggs were found. After 30 hours' incubation, all the eggs had hatched out.

(3) Eggs discharged by a female worm were incubated in normal saline at 28°C. for 77 hours. Only a small proportion of the eggs developed vermiform embryos. A few were hatching and there were a few actively moving free larvae.

(4) 200 eggs discharged by a female *E. vermicularis* in normal saline were kept at 37°C. 19 hours later, 31 per cent. and, 23 hours later, 58 per cent. of the eggs had developed vermiform embryos but no free larvae nor emerging larvae were found. After 122 hours' incubation, 3 per cent. empty shells, 14 per cent. hatching and 83 per cent. unhatched eggs were found.

(5) A few eggs containing vermiform embryos were transferred to different concentrations of saline and incubated at 37°C. for 23 hours. The results were as follows :—

(*a*) In 0.85 per cent. saline, 7 free larvae were found and 3 of them were moving actively.

(*b*) In 0.65 per cent. saline, 11 free larvae were found and 2 of them were moving actively.

(*c*) In 0.45 per cent. saline, 27 free larvae were found ; 3 of them were active.

(*d*) In 0.25 per cent. saline, 11 free larvae were found ; 3 of them were active.

(6) Eggs containing vermiform embryos were used to carry out the following experiments :

(*a*) The eggs were transferred to 50 per cent. artificial gastric juice in distilled water and incubated at 37°C. for 17 hours. All of them hatched, producing free larvae which moved actively.

(*b*) The eggs were transferred to N/50 hydrochloric acid solution and incubated at 37°C. for 16 hours. None of them hatched out but after 64 hours' incubation, 7 free larvae were found.

(*c*) The eggs were transferred to N/25 hydrochloric acid solution. 16 hours later, no free larva was found. After 64 hours' incubation at 37°C. only 1 free larva was found.

The above experiments (6*b* and 6*c*) on the hatching of the eggs of *E. vermicularis* in dilute hydrochloric acid solution were repeated. On this occasion, the eggs were discharged in water and incubated at 37°C. for one night but no development of vermiform embryos took place. On transferring them to a piece of wet filter paper and incubating for another night, embryos developed in a few eggs. These were transferred to two dilute hydrochloric acid solutions (N/50 and N/25) and incubated at 37°C. 23 hours later, 17 free larvae were found in the first solution and 34 in the second. None of the larvae were motile.

(7) Eggs containing vermiform embryos were transferred to a 0.5 per cent. sodium bicarbonate solution and incubated at 37°C. for 17 hours. One free but inactive larva was found and several eggs were in the act of hatching. The experiment was repeated under the same conditions except that the incubation period was 23 hours. Ten free larvae were found but they were not moving.

(8) Eggs containing vermiform embryos were transferred to a 0.25 per cent. sodium bicarbonate solution and incubated at 37°C. for 17 hours. Five free larvae were found but they had no movement.

(9) Eggs containing vermiform embryos were transferred to human sweat and incubated at 37°C. for 48 hours. A few emerging larvae were found but no free larvae.

(10) Eggs containing vermiform embryos were transferred to human serum and incubated at 37°C. for 17 hours. No larvae hatched out. After 73 hours' and 93 hours' incubation, the embryos were still in their shells. The experiment was repeated and gave the same result.

(11) Eggs containing vermiform embryos were transferred to 10 per cent. albumin in water and incubated at 37°C. for 5 hours. Two free larvae were moving actively. 23 hours' incubation produced 9 free and 3 emerging larvae. The larvae lived for 18 hours.

(12) To determine whether eggs contaminated with urine could hatch out or not, we carried out the following experiment. Eggs

containing vermiform embryos were transferred to a slide and dried for three hours. They were then moistened with several drops of urine and the slide was put in a Petri dish containing several c.c. of water to prevent the evaporation of urine. After incubation at 37°C. overnight, three emerging larvae were found. Two days' incubation produced one free and three emerging larvae. Many of the eggs did not hatch.

(13) Segmented eggs discharged by a gravid female *E. vermicularis* were transferred to a piece of cellophane. This was placed on the right inguinal region of the author, so that the eggs were in contact with the skin. The cellophane was then covered by some cotton wool and a piece of paper, which were held in place by three strips of adhesive plaster and left for 24 hours. The cellophane was then removed and a piece of Scotch tape was applied to the area to recover the eggs from the skin. To prevent infection, the skin was sterilized with alcohol. Eggs containing vermiform embryos were found in both the cellophane and Scotch tape but there was no evidence of emergence nor of free larvae.

Experiments on Retrofection in *Aspiculuris tetraptera*.

The hatching of the eggs of A. tetraptera *on a moist cotton wool swab.*

The hatching of the eggs of *A. tetraptera* in different solutions has been demonstrated above. It was of interest to ascertain also whether or not they can hatch out on a moist surface, so a series of tests was planned to investigate this problem. An NIH swab tube was used in which the cellophane was replaced by a piece of cotton wool at the end of the glass rod. It was moistened with a drop of 0.25 per cent. saline and kept moist by about 3 c.c. of water which was poured into the test tube. Eggs of *A. tetraptera*, just isolated from mice faeces, were transferred to the cotton wool swab, which was then replaced in the test tube but was not in contact with the water. In all, four tubes were prepared and incubated at 37°C. After 24 hours about 1 c.c. of normal saline was dropped on a large slide and the cotton wool swab from one of the tubes was gently stirred in it to wash the eggs on to the slide. When examined under a binocular microscope it was found that the eggs had developed to the vermiform embryo stage but no free larvae had hatched. After 48 hours the second tube was examined. 3 free larvae, 17 emerging larvae, 3 empty shells, 29 embryonated eggs and 3 unembryonated eggs were found. After 72 hours, the third tube was examined and 13 free larvae, 30 emerging

larvae, 12 empty shells, 6 embryonated eggs and 10 unembryonated eggs were found. After 96 hours the fourth tube was examined and 13 free larvae, 31 emergences, 13 empty shells, 60 embryonated eggs and 3 unembryonated eggs were found.

The hatching of the eggs of A. tetraptera *on human skin.*

Although the eggs of *A. tetraptera* were thus shown to be able to hatch out on a moist cotton wool swab, it was still not certain whether or not they could hatch on moist skin. Three experiments were carried out to ascertain this.

Experiment 1.

About 40 segmented eggs of *A. tetraptera* were applied to the left forearm of the author and were covered by a piece of cellophane which was overlapped by a piece of cotton wool. These were held in position with adhesive plaster. 18 hours later embryos had developed in the shells but no free larva was found.

Experiment 2.

Eggs of *A. tetraptera* (recently isolated from mice faeces) were transferred to a piece of cellophane by means of a loop. In some of them vermiform embryos had formed, while others were still in the segmented stage. No free larvae were present. The cellophane was then attached to the right inguinal region of the author, covered by a piece of cotton wool and held in position with adhesive plaster. 42 hours later, 75 eggs were found on the cellophane but there were no free larvae. Some eggs had a degenerate appearance. Any remaining eggs or larvae adhering to the skin were removed by means of Scotch tape. 40 eggs but no free larvae or empty shells were found in this way.

As a control, other eggs from the same batch as used in the above experiment were applied to a cotton wool swab moistened with saline. They were incubated at 37°C. and 42 hours later they had hatched.

A third experiment was carried out, using the same method except that the eggs were applied to the skin for an extended period of 67 hours. The results were also negative.

The hatching of the eggs of A. tetraptera *in a human anal opening.*

As shown above, the eggs of *A. tetraptera* would not hatch on human skin either of the forearm or of the inguinal region. It was thought possible, however, that they might hatch in the anal region. A series of experiments were carried out to determine this.

Experiment 1.

Eggs of *A. tetraptera* were isolated from mice faeces and cultured in water at 24°C. for 4 days. 371 eggs were transferred to a piece of cellophane, which was inserted into the anal opening of the author at 12 noon on January 6th, 1950. 22 hours later the cellophane was taken out and put on a drop of water on a slide. Another drop was added to the surface of the cellophane and covered by a coverglass. On examination, three eggs of *A. tetraptera* were found, one with a vermiform embryo which had degenerated to half the normal size, while the other two had a normal appearance.

Experiment 2.

In the second experiment, eggs of *A. tetraptera* were cultured at 25°C. for 66 hours. A large number of them was transferred to a piece of cellophane and examined under a binocular microscope. No free larvae or emerging larvae were found. The cellophane was inserted into the anal opening of the author. A small number of the same batch of eggs was transferred to 0.25 per cent. saline and incubated at 37°C. as a control. 24 hours later the cellophane was removed. The anal region was swabbed by an NIH swab and any remaining eggs or larvae were removed by a piece of Scotch tape. A few drops of 70 per cent. alcohol were put on a slide. The cellophane was put into 70 per cent. alcohol on a slide, covered with a coverglass and examined. One larva, 2 emerging larvae, 2 empty shells, 13 eggs containing normal vermiform embryos and 1 egg with a degenerated embryo were found. On the NIH swab there were 2 empty shells and 2 emerging larvae, and on the Scotch tape swab 2 empty shells and 8 eggs. In the control batch of eggs kept in 0.25 per cent. saline there were several free larvae.

Experiment 3.

Eggs of *A. tetraptera* were isolated from mice faeces and cultured in water at 25°C. for one night, after which time they developed vermiform embryos. They were transferred to a piece of cellophane and examined to ensure that no free larvae nor emerging larvae were present. The cellophane was inserted into the anal opening of the same volunteer and 24 hours later it was taken out and put in 10 per cent. formalin solution. Any other eggs or larvae remaining adhered to the skin were removed by means of Scotch tape. 12 empty shells and 30 eggs of *A. tetraptera* were found on the cellophane and 19 eggs on the Scotch tape.

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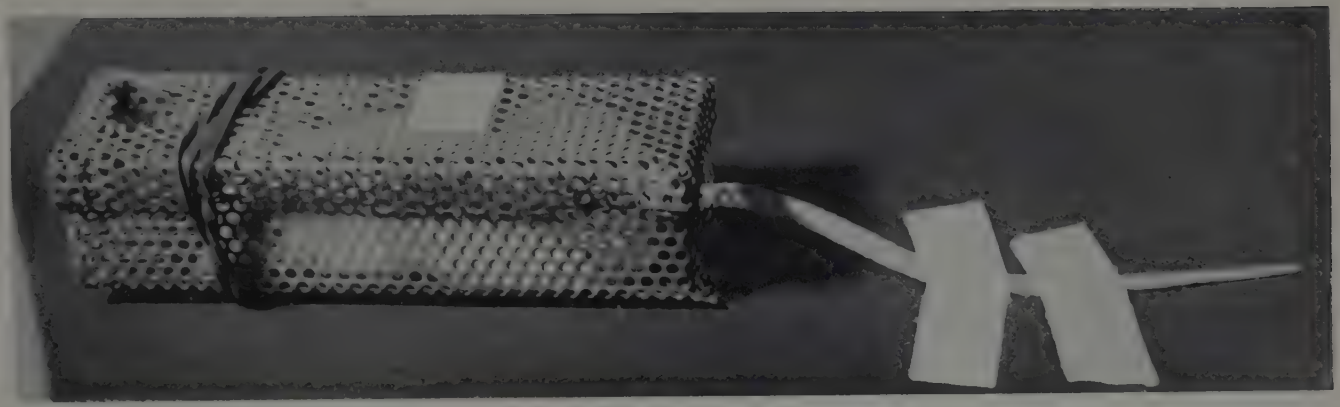

Fig 1 The cage used for immobilizing mice in retrofection experiments.

Experimental demonstration of retrofection in A. tetraptera.

To investigate the possible occurrence of retrofection of *A. tetraptera* in a mouse, a special cage was designed (Fig. 1) to immobilize and to prevent it licking its anal region. The cage was one inch in height and width, four inches in length and was made of perforated zinc with a lid of the same material. At the front part of the lid, two holes were made to supply food and water. A funnel containing wheat was connected with one hole in the cage so that the mouse could eat continually, and a tube of water was connected with the other hole. A hole was made at the bottom of the back of the cage so that the faeces of the mouse could drop out, and another one at the front for cleaning out waste food. At the back of the cage a small hole, through which the tail extended, was made. The cage was fixed down on a wooden base and the tail of the mouse was also stuck to it by a piece of adhesive plaster. In this way the mouse was immobilized but comfortably housed.

Experiment 1.

Eggs of *A. tetraptera* were isolated from the faeces of six infected mice and incubated in distilled water at 25°C. for 19 hours, after which time they contained larvae but the larvae had not emerged from their shells. On January 12th, 1950, at 12 noon, a large number of these eggs was applied to the anal region and vagina of a mouse (No. 18) whose faeces had been repeatedly examined both by direct smear and by floatation technique for one month and had proved negative for *A. tetraptera* eggs. The mouse had been starved for 18 hours to reduce its bowel movement and during the experiment it was immobilized for 46 hours in the special cage described above, which had been sterilized in boiling water for 10 minutes. It was then killed and its anal region was dissected out for examination. The anus was washed in a small quantity of 10 per cent. formalin which was examined under a microscope. Two free larvae, one empty shell and one egg were found. The colon was opened in a Petri dish containing several c.c. of normal saline. Three larvae of *A. tetraptera* were found which measured as follows :

Length, μ	*Width*, μ
148	17
148.75	19.12
144.5	17

The caecum, small intestine, stomach and oesophagus were negative for parasites. The vagina, uterus and bladder were also negative.

Experiment 2.

Eggs of *A. tetraptera* were isolated from the faeces of six infected mice and cultured at 25°C. for 18 hours.

Mouse No. 16 was kept in a round glass cage from December 22nd, 1949, onwards and its faeces were repeatedly examined both by direct smear and by floatation technique. It was proved negative for parasitic eggs. The same mouse was starved from January 16th, 1950, at 5 p.m., for 17 hours to avoid the discharge of large amounts of faeces. On January 17th, at 10 a.m., a large number of the eggs of *A. tetraptera* prepared as above were applied by a loop to the anal region of this same mouse which was immobilized in the cage already described. It was fed on small amounts of wheat and water for 74 hours and finally killed by chloroform.

The digestive tract was carefully examined. From the colon, five larvae were recovered which measured as follows :

Length, μ	*Width*, μ
144.5	17
144	17
148	21.2
157.25	21.25
212	25.5

In the caecum one larva was found which measured 180μ by 22.2μ.

The anus, rectum, small intestine and stomach were examined but all were negative.

Experiment 3.

Mouse No. 20 was kept in a small round cage from January 27th, 1950, onwards. Its faeces were repeatedly examined both by direct smear and by floatation technique and it proved negative for any parasitic eggs. From 5 p.m. on March 6th, 1950, it was starved for 18 hours. Its anal region was then wetted with 0.25 per cent. saline and a large number of eggs of *A. tetraptera* were applied to the anus by means of a loop. The eggs had been isolated from the faeces of six infected mice. Some of them were cultured at 25°C. for 180 hours and the others at 37°C. for 18 hours. After applying the eggs to the anal region, the mouse was immobilized by the technique described above. It was fed on wheat and water for 48 hours and then killed

by chloroform. On examining the digestive tract carefully, two larvae were found in the colon and their measurements were as follows :

(1) Length 135μ ; width 17μ ; oesophagus and bulb 51μ.

(2) Length 147μ ; width 15μ ; oesophagus and bulb 59.5μ.

The caecum contained a single larva whose measurements were :

Length 153μ ; width 19.125μ ; oesophagus and bulb 57.375μ.

The rectum, small intestine and stomach were carefully examined but with negative results. From the anus, one egg of *A. tetraptera* was recovered.

Experiment 4.

Mouse No. 8 was fed in a small glass cage from September 22nd, 1949, onwards. Its faeces were collected weekly for examination by direct smear and floatation method. It was proved to be free of parasitic eggs. On November 14th, it was immobilized in a cage and 3,000 eggs of *A. tetraptera* which had been cultured in water at 25°C. for 6 days were injected into the anus.

On November 15th its faeces were examined by direct smear and one egg of *A. tetraptera* was found. On November 16th all the faeces discharged by the mouse were collected and examined by floatation method, and eight eggs of *A. tetraptera* were recovered. On November 18th two eggs were recovered from the faeces by the floatation method. Subsequent examinations were negative. The vermiform embryos of these eggs appeared to be viable and no empty shells were found.

On the morning of November 28th the mouse was found to have jammed itself in the cage in attempting to turn round. When it was released it was in a critical condition. It was therefore removed to a large round glass cage and three hours later it had recovered.

On November 29th it was chloroformed and its abdominal wall was opened. The alimentary canal was removed and the contents of the oesophagus, stomach, small intestine, caecum and colon were examined separately. In the colon two immature *A. tetraptera* were found which measured as follows :

(1) Length 1152μ ; width 102μ ; Length of oesophagus 276.25μ ; Length of tail (from anus to the posterior end) 140.25μ.

(2) Length 1116μ ; width 97.75μ ; Length of oesophagus 165.75μ ; Length of tail 140.25μ.

Experiment 5.

Mouse No. 15 was kept in a round glass cage from December 22nd, 1949, onwards. Its faeces were examined every week by both direct smear and floatation technique and proved to be negative. On January 24th, 1950, 430 eggs of *A. tetraptera* were applied to its anal region. These eggs had been isolated on January 23rd and incubated at 25°C. for one night. After being immobilized for four days in the special cage the anal region and the hairs on the buttock, abdomen and posterior legs were sterilized with a 10 per cent. formalin solution and the mouse then released and fed in a glass cage. Its faeces were examined but no egg was found up to April 3rd. On April 4th, after 18 hours' starvation, a large number of eggs of *A. tetraptera* were again applied to the anal region of the same mouse and it was immobilized in the special cage. The eggs used for this experiment had been isolated from the faeces of six infected mice and incubated at 37°C. for 18 hours. The special cage used to immobilize the mouse had been sterilized in boiling water for ten minutes.

In the special cage it was fed with oats and its appetite was very good. On April 6th it was released and fed in the glass cage after being cleansed with formalin as described above. Up to April 28th, its faeces were negative but on May 5th two *A. tetraptera* eggs were recovered.

On May 8th, the mouse was chloroformed and the colon, caecum, small intestine and stomach were carefully examined. Six adult female worms of *A. tetraptera* were found in the colon ; no other parasites were present. They measured as follows :

Length, μ	*Width*, μ	*Oesophagus*, μ
3726	252	450
3726	261	426
3672	243	441
3366	243	432
3600	252	468
3078	225	450

Experiment 6.

Mouse No. 40 was kept in a sterilized round glass cage from March 16th, 1950, onwards. Its faeces were examined weekly both by direct smear and by floatation technique and proved negative. On April 12th, at 1.30 p.m., *A. tetraptera* eggs which had been incubated at 37°C. for 18 hours were applied to the anal region of the mouse which was immobilized for three days. On April 15th, its anal region, the hairs of

abdomen, buttock and hind legs were sterilized with 10 per cent. formalin and it was then transferred to the round glass cage.

On May 12th, one egg of *A. tetraptera* was found in its faeces. On May 15th, the mouse was chloroformed and its alimentary tract was carefully examined. From the colon three adult female *A. tetraptera* were recovered. One of them had been cut into two pieces during opening of the intestine. The other two measured as follows :

(1) Length 3906μ ; width 180μ ; length of oesophagus 414μ.
(2) Length 4041μ ; width 207μ ; length of oesophagus 441μ.

Experiment 7.

On March 16th, 1950, mouse No. 41 was isolated in a sterilized round glass cage. Its faeces were examined weekly by both direct smear and floatation technique and proved negative. On April 12th, eggs of *A. tetraptera* isolated from mice faeces and incubated at 37°C. for 18 hours, were applied to the anal region and the mouse was immobilized. On April 15th, it was released from the special cage and after sterilizing its anal region, the hairs of abdomen, buttock and hind legs with 10 per cent. formalin, it was transferred to a round glass cage. On May 16th, four eggs of *A. tetraptera* were found in its faeces. The mouse was chloroformed and its colon, caecum, small intestine and stomach were examined. From the colon, four female and two male *A. tetraptera* were recovered. They measured as follows :

Females :

Length, μ	*Width*, μ	*Oesophagus*, μ
4176	199.8	414
4122	201.6	395
3906	216.0	432
3249	162.0	387

Males :

3141	144.0	315
3204	158.0	360

Control for the experiments on retrofection in Aspiculuris tetraptera.

From March 7th, 1950, onwards, mouse No. 35 was isolated in a sterilized round glass cage. Its faeces were examined weekly by both direct smear and floatation technique. No egg was found. On May 18th, the mouse was chloroformed and its colon, caecum, intestine and stomach were carefully examined but no parasite was found.

Although only one mouse was used as control in this experiment the following six mice with negative results, in spite of experimental attempts to induce retrofection, may be regarded as additional data. On six occasions eggs in the infective stage of *A. tetraptera* were applied to the anal region of mice (Nos. 10, 12, 17, 21, 45, 56) which had been examined for a period ranging from 4 to 12 weeks and proved negative to natural infection of *A. tetraptera*. Three of them were killed to investigate whether larvae of *A. tetraptera* were present after 2 to 7 days' application of eggs, but no larvae or adults were found in the alimentary canal. Subsequent examination of the faeces of the other three mice for eggs were negative. These mice were killed at the end of 5 weeks or more and the alimentary tract contained no larvae or adults.

Summary.

1. Observations were made on the development and hatching of eggs of *Aspiculuris tetraptera* in different media, preparatory to experiments investigating the possible occurrence of retrofection in this Oxyurid. Eggs of *Enterobius vermicularis* were studied under similar conditions.

2. Infective eggs of *A. tetraptera* were applied to the anal opening of clean mice which were immobilized in cages specially designed to prevent the possibility of oral infection. Of 12 mice treated thus evidence was obtained from 6, by the finding of immature or adult worms in the intestine, that retrofection had taken place. This constitutes the first experimental proof of the occurrence of retrofection in a non-human Oxyurid.

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The Use of a Microbalance in Putting up Uniformly Sized Batches of *Heterodera* Cysts for Experiment.

By D. W. Fenwick, M.Sc. and Elizabeth Reid.

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When setting up experiments involving hatching tests on cysts of *Heterodera rostochiensis*, the high degree of variability inherent in the material is a serious complicating factor. Fenwick (1950) found that the coefficient of variability for the larval content of cysts was of the order of 21%, and 10·5% for batches of 25 and 100 cysts respectively. He therefore recommended the use of batches of at least 50 and preferably 100 cysts, and stressed the need for adequate replication of batches. Further experience has convinced the authors that for experiments involving up to twenty subtreatments, at least five fold replication is desirable. Setting up a large scale experiment with this high degree of replication becomes a somewhat lengthy and tedious procedure. A technique saving time on this operation would therefore be of value.

Lownsberry (1950) obtained cyst samples of equal number by weighing, and it appeared to the authors that if resultant loss in accuracy were not excessive, weighing might be very convenient. A microbalance was therefore constructed and experiments carried out to investigate the magnitude of the errors introduced as a result of its use.

Apparatus and Technique.

The microbalance consists of a glass tube drawn out to a fine capillary about 18 in. long, the end being bent into a small hook to support a small metal pan. A photograph of the complete apparatus and a close-up of the pan and hook is given in Plate 1. The tube is mounted on a heavy brass rod pivoted at its centre. Coarse adjustment of the angle of the arm is possible by means of the elbow joint to the left of the support, and fine adjustment can be effected by the knurled screws on either side of the pivot. Behind the distal end of the arm is a vertical millimetre scale mounted on a mirror, so that parallax errors can be eliminated. A movable horizontal arm

operated by a rack and pinion assembly, supports the capillary when the pan is being removed and replaced or when the balance is not in use. The whole apparatus is enclosed in a glass case so that errors due to air currents are eliminated. Before use, the balance, with the pan in position, is adjusted to the zero of the scale and is calibrated by adding known weights to the pan and noting the deflection. In practise the deflection is perfectly linear up to 50 mgm., and sensitivity of the order of 1 mgm. per 2 mm. deflection is easily attainable with a suitable capillary. One capillary has now been in use several months, and although the balance needs periodic adjustment for correction of zero errors no difference in sensitivity has yet been recorded; nor is there any evidence of varying sensitivity with change of temperature. The capillary is, moreover, sufficiently robust to withstand normal treatment without fracture.

The pan in which the cysts are weighed is made from aluminium foil 0·001 in. thickness, by shaping on the end of a slim pencil or similar object. A small strip of metal is left above one side after trimming and in this a hole is pierced approximately $\frac{1}{16}$ in. diameter to fit on to the capillary hook.

The weight of the pan is approximately 14 mgm. and, as the balance is first adjusted to zero with the empty pan in place, the weight of cysts inside can be read off, directly.

The cysts for experiment are rolled as thoroughly as possible from material recovered from a large soil washing apparatus (Fenwick, 1940), and ample cysts for the whole experiment are placed in a flat-bottomed 3×1-in. tube. Small metal spoons are used for sampling the cysts. These are made from small pieces of zinc foil and mounted on the end of a heavy gauge bicycle spoke. It is convenient to have a number of differently sized spoons available for weighing out batches of varying size. When sampling the cysts, the tube is held at an angle of 45° and slowly rotated several times to ensure thorough mixing. The spoon is pushed to the bottom of the cyst mass and lifted up through it, thus withdrawing a sample of cysts. These are transferred to the balance pan for weighing. Any samples falling outside the arbitrary limits of ±10% around the required weight, are discarded, and all individual weights noted.

Using a suitable spoon of convenient size and observing reasonable care, only rarely does the weight of a batch of cysts fall outside these limits.

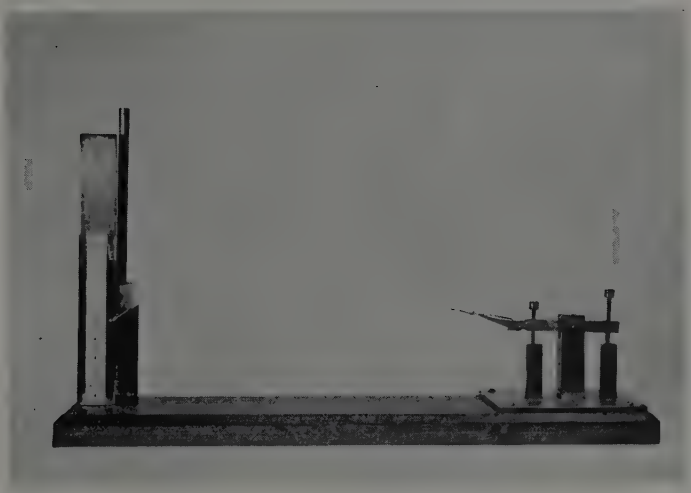

Plate 1.

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A preliminary experiment was set up to investigate how the variability in cyst number per sample was influenced by the size of the sample. Samples of cysts of such a weight as to contain approximately 25, 50, 100, 200, 400 and 800 cysts were weighed out in sets of ten. The cysts in each sample were enumerated and the results recorded, together with the sample weight in balance scale units. The mean weight of each duplicate set of samples was estimated and each individual cyst count corrected for recorded weight variations by multiplying it by the mean weight and dividing by its own weight. The mean, and standard deviation of cyst number for each set of ten samples was calculated. This value divided by the mean for each group of ten gave an estimate of the coefficient of variability. The results are set out below.

No. of cysts/sample ...	26	59	108	188	436	805
Coefficient of variation	22·1	9·35	9·60	6·45	6·8	5·8

TABLE I.

Data showing consistency obtained by weighing cysts from different sources.

Cyst type	A	B	C	D	E	F	G	H	I	J
Mean no. of cysts	112·5	114·5	115·4	101·4	112·9	109·1	102·7	112·4	124·7	123·2
σ	9·7	7·3	12·7	6·0	8·0	6·9	7·2	9·2	13·2	11·3
Coefficient of variability	·086	·064	·110	·059	·071	·063	·070	·082	·105	·092

It will be seen that the coefficient of variation decreases as sample size increases. Thus in this experiment a sample containing 25 cysts is subject to an error of 22% of the mean, for 100 cysts about 10% and for 900 cysts it falls to 5%.

A further test was conducted to investigate how far these standards of accuracy applied to cysts from other sources. In all, ten different cyst types were tested. In each case 10 replicate samples containing approximately 100 cysts were weighed out and the number of cysts present in each sample counted. After correction for weight variations, the mean and standard deviation for each cyst type was computed as well as the coefficient of variability. The results are set out in Table 1.

It will be seen that only in two cases does the coefficient of variability exceed 10%, the general value for this being 8·5% for all data. It is reasonable to assume that in general, samples of 100 cysts can be weighed from rolled debris with an accuracy of 10%.

It is to be expected that the variability in cyst number per batch introduced by weighing would result in a corresponding increase in error in the larval count and it was decided to investigate this point. To this end the cyst batches from the previous test were subjected to a larval count. To act as a control 10 further batches were counted from each cyst type containing the mean number of cysts as calculated

TABLE II.

Analysis of variance for larval counts from weighed and counted batches.

Source	*SS*	*D. of F.*	*Mean square*	*Ratio*
Error	63,417	200	$317{\cdot}08v_E$	
Between weighed batches within types	232,138	90	$2579{\cdot}3v_{T_W}$	$v_{T_W}/v_E = 8{\cdot}15$
Between counted batches within types	185,003	90	$2055{\cdot}6v_T$	$v_T/v_E = 6{\cdot}5$

from the weighed batches. The degree of dilution for each cyst type was adjusted so that over the whole experiment the number of larvae counted per batch remained more or less constant. Two 1 ml. samples were examined per batch. The data for the weighed batches were corrected for weight variations as mentioned earlier and the corrected weighed data together with the counted data subjected to an Analysis of Variance given in Table II. It will be seen that the mean square for " between batch " variability has been split up into that ascribable to weighed batches on the one hand and that to counted batches on the other, the corresponding values for the variance of the batch means being 1289·6 and 1027·8 respectively. By subtracting from these values the portion ascribable to error variance estimates of " absolute

between batch " variances are obtained corresponding to 1131 and 869 for weighed and counted data—a ratio of 1·30 which suggests that if similar accuracy is to be attained from both methods 1·3 times the number of replicates must be used for weighed batches as compared with counted.

A further analysis was performed in which an estimate of the " absolute between batch " variance was computed for weighed and counted batches for each cyst type used and in only two cases did the ratio of variances exceed 2·0 ; it is difficult to see how far these high values were a true reflection of an abnormally high variability in view of the fact that the individual variances were each based on only 9 degrees of freedom and a ratio of 2·80 (the maximum value) was barely significant under those conditions. It is, in fact, difficult to avoid drawing the inference that under ordinary conditions of experiment the loss of precision consequent on weighing is scarcely detectable. There can be very little doubt but that if replication is increased by 50% or 100% the degree of precision attained by weighing is at least as great if not greater than by counting.

Summary and Conclusions.

Tests were conducted using a capillary microbalance to estimate the errors introduced as a result of weighing out replicate cyst batches in place of counting.

Experiments with differently sized samples showed that the normal relationship between sample size and accuracy applied to such cases, the error for samples of 100 cysts being usually of the order of less than 10%. There is some evidence that the errors in cyst number introduced as a result of weighing is reflected by an increased error in the larval count, although under the conditions of experiment the increase was barely significant. It is suggested that if replication is increased by 50% or 100% then errors due to inequality in cyst numbers are more than counteracted.

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Journal of Helminthology, Vol. XXV, *Nos.* 3/4, 1951, *pp.* 166—172.

On the Varying Nematicidal Effects of Different Samples of D-D against the Potato-root Eelworm *Heterodera rostochiensis*.

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Although the literature concerning the effect of the soil fumigant, D–D, on the potato-root eelworm under field conditions is already extensive, little if any fundamental work has been done to investigate the effect of this chemical on the parasite under the more easily controlled conditions of pot culture. As far as the author is aware, no attempt has been made to investigate the relationship between dosage of D–D, and the resulting mortality induced in a population of *Heterodera rostochiensis*; no serious attempts have been made to investigate the constancy or otherwise of the substance, even though it is widely known that Shell D–D is a by-product in the manufacture of plastics, and as such may vary widely in its chemical composition. Investigations into this problem based on chemical analysis are unfortunately not practicable owing to the extreme complexity of the mixture; consequently a suitable method of investigation would appear to be a biological one. The possibility that samples might differ from one to the other was supported by the fact that several experiments in which the author was concerned yielded surprisingly contrasting results, which seemed impossible to explain except on the basis of differences between different samples of D–D. An attempt was, therefore, made to investigate the comparative efficacy of different samples of D–D as nematicidal agents.

With this object in view, four samples of D–D taken from four barrels were chosen for experiment. As far as the author is aware, these four barrels may have been drawn from one bulk source of supply. On the other hand, the results of the tests set out later in the present paper would seem to suggest that they may have come from four different batches of material. All that one can say, is that four barrels of material were supplied, and one presumed that the material

was of a fairly constant character. The effect of these samples was tested on a naturally infested soil taken from the kitchen garden at the Institute of Agricultural Parasitology, St. Albans. It was a moderately heavy loam which probably was rather deficient in humus. A mass of this soil was taken, partially dried, then sieved through a ¼-in. mesh riddle ; the sievings were coned and quartered three times, after which they were potted into 5-in. pots. Six doses of D–D were chosen including one control, 0·1, 0·2, 0·4, 0·8 and 1·6 cc. per pot ; this in conjunction with four samples of D–D yielded 24 separate subtreatments. Each subtreatment was represented by five replicate pots making a total of 120 pots in all. The D–D was injected into the centre of each pot, using a glass rod inside a glass tube to penetrate the soil mass ; the D–D was introduced into the tube after withdrawing the rod ; the tube was then withdrawn, the soil pressed together round the hole and the pot given a light watering with a fine rose to seal the surface of the soil. The pots were then left for one calendar month after which the soil from each was washed and the cysts recovered. The injections were carried out in early March of 1948 and the pots during experiment were kept in an unheated greenhouse.

Hatching tests were conducted on the cysts in the following October, one batch of 100 cysts being taken from each pot for experiment. All the usual precautions were observed in selecting the cyst samples and the usual technique applicable to hatching tests on multiple cyst samples was followed. The total number of larvae liberated from each batch was recorded and when hatching was complete, the number of eggs and larvae left in the cysts was counted.

Two methods were used to estimate kill. In the first, the number of larvae hatching out of the cysts in each batch was added to the number left in the cysts after hatching was complete. This was considered to be the best available estimate of the number of eggs and larvae originally present. The number of larvae hatching out in the control batches was then expressed as a proportion of the original total content and was found to be 59·2%. The totals obtained for the treated batches were then multiplied by this factor to obtain an estimate of the number of larvae which would have hatched out of these cysts if they had not been treated with D–D. This corrected total was then taken to represent the number of " hatchable " eggs and larvae exposed to treatment ; the number of larvae hatching expressed as a proportion of this gave the percentage viability after treatment. This was then subtracted from 100 to give the percentage

kill. Examination of the data showed that the estimate of total eggs and larvae originally present fell as dosage was increased, and this decrease with increasing dosage was significant. The effect of this caused over-estimation of the viability after treatment, the degree of error increasing with increased dosage. The other method of estimating kill was to determine the number of larvae which hatched out of the controls and regard these as a measure of the number of larvae which would have hatched out of the experimental batches if they had not been exposed to treatment. All hatches were then expressed as a percentage of the control hatch, this being regarded as an estimate of the after-treatment viability. Again, this value subtracted from 100 gave an estimate of the percentage mortality.

TABLE 1.

Parameters of probit curves for different samples of D–D.

Sample	a	V_a	b	V_b	*D. of F*	χ^2
A	5·60	·0012	0·48	·00075	3	8·98
B	5·66	·0007	0·38	·00024	3	5·40
C	5·55	·0010	0·31	·00057	3	8·30
D	5·70	·0013	0·29	·00060	3	8·90

The data thus obtained were subjected to a straightforward probit analysis as described by Bliss (1935a, 1935b), the data from each sample being analysed separately and the parameters thus obtained being compared with each other in the normal way. For ease of computation the log of dosage was coded to $x = \frac{1 + \log d}{0{\cdot}301}$ where d was the dosage in ccs. Direct comparisons of the constants of each probit line indicated that the mean mortality for all samples over the range of dosages tested fluctuated from 5·55 to 5·70 probits, corresponding to kills of 70·9 and 75·8% respectively; the difference between these values bordered on the $p = {\cdot}05$ level of significance. The values of the statistic b, which governed the slope ranged from 0·29 to 0·48. There was some evidence that these values differed significantly. The summarised data for the four curves are given in Table 1 with the corresponding value χ^2 for each curve : data in this table are recorded

in terms of coded log dosage. This relative constancy in the mean would result in equal quantities of all samples being needed to give a 50–60% mortality. The differences in slope would result in varying

TABLE 2.

Probable dosages with 95% *limits for specified kills using different samples of D–D. Data in ccs. per* 5*-in. pot.*

% *kill*		*A*	*B*	*C*	*D*
	upper limit	6·46	9·8	69·2	72·4
99	probable value	3·25	6·3	22·9	16·6
	lower limit	3·29	4·2	7·76	7·24
	upper limit	1·91	2·3	8·91	7·76
95	probable value	1·38	1·7	3·92	3·16
	lower limit	0·98	1·3	2·29	1·82
	upper limit	1·05	1·1	3·02	2·24
90	probable value	0·76	0·89	1·66	1·35
	lower limit	0·62	0·69	1·10	0·80
	upper limit	0·38	0·35	0·54	0·40
75	probable value	0·32	0·30	0·43	0·30
	lower limit	0·28	0·28	0·33	0·22
	upper limit	0·14	0·11	0·13	0·041
50	probable value	0·12	0·081	0·093	0·059
	lower value	0·089	0·069	0·056	0·030
	upper limit	0·068	0·035	0·035	0·021
25	probable value	0·046	0·026	0·020	0·012
	lower value	0·033	0·017	0·008	0·004

amounts of different samples being needed for very high or very low kills, the samples which appeared to be most lethal at low concentrations would be the least lethal at high.

To investigate this for the samples of D–D under experiment, arbitrary mortalities of 25, 50, 75, 90, 95 and 99% were considered and the probable dosage of each sample necessary to give each kill was estimated together with its standard error. The results of this are set out in Table 2. The upper and lower limits given in the body of this table refer to the experimental limits of error in the determination corresponding to $p = \cdot 05$, which means that when two ranges do not overlap, the difference between the probable values for each range is highly significant. It will be seen that at 25% kill the lethality decreases from D to A. The differences become less pronounced as kills approach 50–75% ; above these values the order of lethalities is reversed, A being most lethal while D and C are the least potent. It is interesting to note that all through the experiment D appears to be more efficacious than C due to the fact that the probit lines for both seem to have the same slope but the mean lethality for D appears to be slightly higher than that for C. A graphical presentation of the varying comparative quantities of different samples needed to induce these mortalities is given in Fig. 1. It will be seen that at 25% mortality, D is significantly more lethal than is A ; at 90% mortality a significant difference is again established between A and C, but in the reverse direction, while at 99% both D and C require a higher concentration than A.

That the differences in potency of the different samples are not merely of academic interest will be realised when it is remembered that dosage in Fig. 1 is plotted on a logarithmic scale ; to obtain a 99% mortality with sample C would require a probable dosage of 22·9 cc. but the same mortality could be attained with sample A for a dosage of 3·26 cc.—a dosage of about one-seventh of that needed by C. At the other end of the mortality scale, a mortality of 25% can be attained, using a dosage of ·012 cc. of D, but 0·46 cc. of A will be needed to get the same effect—over three times as much.

While the author does not consider himself competent to put forward hypotheses to explain these discrepancies, he would nevertheless point out that they are fully consistent with the presence of a number of different toxic substances in different proportions. This, taken in conjunction with the fact that D–D is itself a mixture, the exact composition of which is unknown, and that this mixture is, moreover, a by-product, would indicate the need for a more fundamental approach to the problem of its use as a nematicide. The fact that the four samples tested were about equal in their nematicidal properties at 50% mortality is no guarantee that all other samples would be equally constant

at this mortality level. It is doubtful, anyway, whether constancy in this region of mortality is of any practical importance in nematode control, since it is improbable that kills of this order have an appreciable effect on the final cyst population when treatment is followed by the growth of a crop of potatoes. Data presented by Chitwood and Feldmesser (1948) indicate that a mortality of 90% results in only a

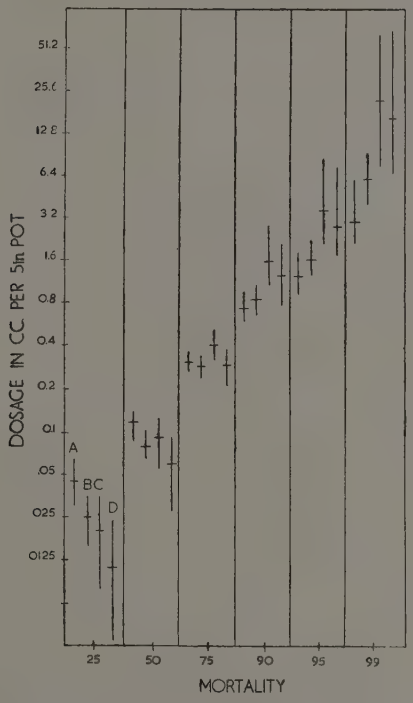

Fig. 1.—Dosage for different samples of D–D for varying mortality levels.

49% reduction in the formation of new cysts. They conclude that a reduction of 99% in viability is needed in order to permit the growing of two crops of potatoes without nematode damage. It is at these high mortalities that the maximum differences in nematicidal activity are exhibited by different samples of D–D.

SUMMARY.

Four samples of D-D were tested at six dosage levels for their nematicidal activity against the potato-root eelworm *Heterodera rostochiensis*; the treatments were carried out on naturally infested soil in 5-in. pots, viability being estimated by hatching in root diffusate. There was no evidence of there being significant differences between the samples at 50–60% kills, but at higher and lower kills they did appear to differ appreciably from one another in nematicidal activity.

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A New Modification of the McMaster Slide for Use in Potato-root Eelworm Investigations.

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The original McMaster slide (Gordon and Whitlock, 1939) has been extensively used for investigations involving dilution counts on helminth ova in faeces. In view of the simplicity inherent in its design and use, attempts have been made to utilize it for obtaining estimates of the viable egg contents of cysts of *Heterodera rostochiensis*, the use of a modified McMaster slide for this purpose being described by Fenwick (1942). Fenwick's slide differed from the original in that the counting area was marked on the floor of the counting chamber instead of its roof ; this modification resulted from the use of water as a diluent instead of semi-saturated brine as used in faecal examinations. The capacity of the original McMaster slide (0·15 ml.) was, however, found to be too small to give a reasonably large count from a batch of 100 cysts unless the degree of dilution was so small that efficient agitation before the withdrawal of a sample for counting was difficult. This difficulty was overcome by a colleague of the author, who uses a slide similar to Fenwick's modification except that its depth is 0·167 cm. and its counting area 2 × 3 cm. further subdivided into 0·5 mm. squares ; the volume of fluid thus examined being 1 ml. Whilst this slide is an improvement on the original it suffers in the writer's opinion from the disadvantage that unless great care is exercised in handling a loaded slide, there is a danger of losing some of the fluid from the chamber due to the lack of side support for the comparatively large volume of fluid contained in it. Moreover, this lack of fluid stability renders it impracticable to mount more than one cell on a single slide. The new design of slide herein described affords complete security to its contained fluid, and five or more chambers can be accommodated in a single unit, thus considerably expediting the counting of a large number of samples.

The new design differs from the old in that the distance pieces (represented by the shaded areas in Figs. 1a and 1b) which determine the depth of the counting chamber, completely surround the latter. Two types of roof are possible for this chamber—it can either completely enclose the chamber as in (*a*) in which case three vents $\frac{1}{8}$ in. diameter are bored at one end and a single charging hole $\frac{3}{16}$ in. diameter at the other ; alternatively it can extend from one edge of the chamber to within $\frac{1}{4}$ in. of the other end, as in (*b*), in which case three vents of $\frac{1}{8}$ in. diameter must be bored at the closed end. To charge the slide it is placed on the bench with the charging holes or slots nearest the operator ; it is convenient in the case of type (*a*) to rest the other side of the slide on a strip of wood or other material about $\frac{1}{8}$ in. thick ; this is unnecessary for type (*b*). The suspension of larvae is run in through either the charging hole or the slot until it completely fills the chamber. Any excess of fluid emerging through the air holes can then be removed by means of filter paper. The slide is now ready for examination and capable of withstanding some considerable degree of jolting without disturbing or losing any of its contents, although type (*b*) is inferior to (*a*) in this respect.

After use the slide can be emptied by turning it on its side when it will drain. The counting chamber can be cleaned by directing a stream of water from a tap into the charging hole ; the efficiency of this method is demonstrated by the jets of water emerging from the air holes. If fluids containing large quantities of organic débris are being examined type (*b*) slide is more satisfactory, since a pipe-cleaner can be introduced into the counting chamber and used as a brush. After thorough washing, excess water can be removed by holding the slide on its side with the air holes lowermost and tapping sharply a few times on to a glass cloth or duster.

Either design can be made up very easily in the laboratory from sheet perspex. The distance piece can be made by cutting five round-cornered windows each of 3 × 5 cm. in an 18 × 6 cm. sheet of $\frac{1}{16}$-in. perspex as in the shaded area of the diagrams. It is advisable to check the thickness of the sheet used with a micrometer, as different samples tend to vary. Theoretically, $\frac{1}{16}$-in. perspex should be 0·159 cm. thick i.e., 4·8% thinner than necessary ; in most instances this does not matter a great deal since a correction factor can be applied to the final results if absolute counts are needed. If desired, the error can be corrected in the preparation of the slide by selecting a strip of perspex which is unusually thick—no difficulty has been experienced by the

author in obtaining pieces of allegedly $\frac{1}{16}$-in. perspex which achieve a thickness of 0·163 cm. when the error falls to 2·4%; alternatively, true $\frac{1}{16}$-in. perspex can be used and the extra ·008 mm. depth can be achieved by sandwiching 44 S.W.G. bare copper wire between the distance piece and the roof when cementing on the latter. The roof

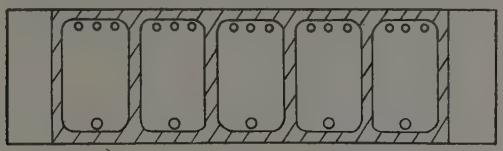

Fig. 1A.

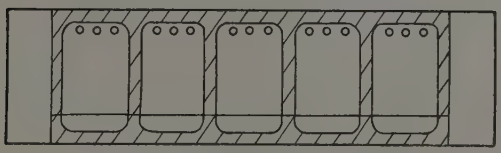

Fig. 1B.

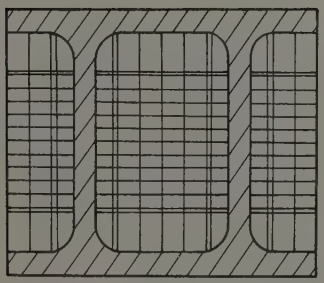

Fig. 2.

of the chamber, consisting of a $\frac{3}{32}$-in. or $\frac{1}{8}$-in. perspex, is now stuck on to the distance piece with "Durofix" glue, the two being clamped together until the glue is set. The holes are then bored in the roof, care being taken that they are set as closely as possible to the ends of each chamber. The base consists of a 6 × 20 cm. sheet of $\frac{1}{8}$-in. perspex

on which the counting areas are inscribed. A convenient method for marking out the latter is to stick on to the base a sheet of millimetre graph paper ; the centre of the paper is then cut out, leaving only a 1 cm. wide border all round the slide. The lines can then be drawn, using a fine needle and straight edge, the markings on the graph paper border ensuring accuracy and rectilinearity. The author finds it convenient to divide each 3 × 2 cm. counting area longitudinally into ten 3 mm. strips and transversely into four 5 mm. sections, the 3 mm. strips being a convenient width for examination with a 40 mm. objective in conjunction with the 10x eyepiece of a binocular microscope. Since lines inscribed by this method are continuous and do not terminate at the margin of the counting areas, it is advisable to inscribe further indicator lines outside the boundaries of the counting areas at 1 mm. distance from them to avoid confusion ; the pattern of a single counting chamber thus appears as in Fig. 2.

Extensive experiments have been conducted to test this slide. Counts from different samples of a suspension of *Heterodera* eggs and larvae have been found to conform to a Poisson distribution. Counts have also been made for the number of eggs and larvae in the different sections of each counting chamber and it has been found that differences over the whole counting area are purely random, there being no evidence of any systematic variation in density capable of correlation with position.

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The Seasonal Variations in the Worm Burden of Scottish Hill Sheep.

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In previous papers (Morgan and Sloan, 1947 ; Cushnie and White, 1948 ; Morgan, Parnell and Rayski, 1950) it has been shown that the number of helminth eggs passed in the faeces of Scottish hill sheep generally follows a distinct seasonal pattern. From a low level in January the number of worm eggs passed reaches a peak about May or early June and then falls gradually throughout the remainder of the year. Occasionally there may be indications of a slight rise in early autumn, or at least a slight delay in the gradual fall of worm egg output, but this is never so marked nor is it so universal in its occurrence as the rise in the spring. Graph 1, which illustrates the general pattern, is based on worm egg counts from hill sheep collected in many districts of Scotland from 1946 to 1950, and shows the " average " worm egg output of Scottish hill sheep from soon after birth until they are sold off the hill as draft ewes.

While this graph shows very clearly the sharp rise in the worm egg output in the spring and its subsequent fall, no undue significance is attached to the variation in height reached at the peak for the different age groups. This graph also shows that in lambs there is, of course, an upward rise from birth to a peak in the autumn.

As already indicated by the present authors in their previous paper (1950) the spring increase in worm eggs had also been noted by several other workers in sheep kept under varied methods of management and climatic conditions. Speculations on the cause of this sharp increase in worm egg output, which occurs at a time when the conditions for the rapid development of the free living stages of nematodes are far from ideal, have tended to the view that it could not be the result of a corresponding increase in the worm burden at that time of the year.

Thus Taylor (1935) concluded that " The rise and fall in the numbers of eggs passed by the ewes does not appear to synchronise with the rise and fall in the intake of infective larvae " . . . " and it is suggested that the fluctuations in egg counts are referable to variations in the rate of egg production of the adult worms rather than to fluctuations in the rate of intake of infective larvae." Naerland (1949) also states " The spring rise in egg output by poorly wintered lambs is assumed to be due to an increased egg producing activity of the remaining females and, probably to a certain extent to new females having developed to maturity from larvae which since the foregoing pasture season have been latently in the intestinal mucosa or perhaps elsewhere now finding the conditions of the host suitable for their requirements." In this country Cushnie and White (1948), who carried out fortnightly counts on a group of sheep, obtained a sharp rise in worm egg counts in March when the ground was still covered with snow. They also concluded that " The increased faeces egg counts in spring arise from greater egg laying activity by worms already present rather than from new infestations."

From what is known of the bionomics of the free-living stages of nematodes it is natural to conclude that the amount of infective material available on pastures in the early months of the year would preclude any marked increase in the worm burden at that time. Indeed Crofton (1949) has shown that the number of infective larvae on hill pastures increases gradually through the summer to a peak about the end of August. It is true that this can be explained by the fact that during the summer there is a big increase in the sheep population on the hills resulting from the lamb crop, and from spring to late summer, a hill flock contains a large proportion of young animals highly susceptible to helminths. Therefore both ewes and lambs are exposed to the highest concentration of infective larvae in summer and early autumn. There is however, at least in some areas, a greater tendency for hill sheep to concentrate on small green patches of pasture in the spring ; this results in local overstocking and the danger of heavy infestations.

The suggestions that the spring increase in the worm egg output results from increased egg laying of worms which have been acquired in the previous summer and autumn does not, however, account for all the facts relating to helminthiasis in hill sheep in the spring. A common occurrence in hoggs is an outbreak, usually in February or March, of what is called " winter trichostrongylosis." when heavy infestations, particularly with *Trichostrongylus* spp. and *Ostertagia* spp.,

occur. In some post mortem examinations carried out on such animals the present authors have seen ample evidence, in the presence of thousands of young stages, to suggest that the infestations were of recent origin. In one outbreak of this disease the pasture was still mainly covered with snow and only a few wind swept patches were available for grazing.

It seemed, therefore, that even in winter, there might be a sufficient number of larvae available to produce heavy infestations and that the spring increase in worm eggs might be due to an increase in the worm burden at that time.

The present paper is an account of an investigation carried out in order to obtain further information on the cause of the spring rise in the worm egg output ; a problem which has an important bearing on the understanding of host-parasite relationships under grazing conditions and on the practical question of prophylactic anthelmintic treatment.

In order to find out whether there is a relationship between the number of eggs passed in the faeces and the number of worms present in the alimentary canal it was decided that sheep would have to be slaughtered at different times throughout a whole year, so that a thorough quantitative and qualitative study could be made of their worm burden.

Material and Methods.

Investigations in 1948–49.—A heft* and a half consisting of 220 Cheviot sheep were bought from Mr. A. Linton, Gilmanscleuch, Ettrick, in August, 1948, for this investigation. The sheep were grazing on the same hill farm as the heft on which continuous observations on the seasonal variations on worm egg output had been in progress since 1944. (Morgan and Sloan, 1947 ; Morgan, Parnell and Rayski, 1950.)

A preliminary study of each of the purchased sheep showed that the level of the worm egg output did not vary greatly from that of the sheep sampled on this farm in previous years. Worm egg counts were made periodically on the sheep selected for slaughter and on those of the neighbouring heft ; the results showed no marked difference between the two groups.

* A heft of sheep is a part of a hirsel or flock, usually a few score, which has the exclusive grazing of a well defined section of hill and which does not stray from that part of the hill. The common breeds of hill sheep in Scotland are the Scottish Blackface and the Cheviot.

It was difficult to decide on the best times of the year for slaughtering and on the minimum number of sheep which would be required at each slaughtering to give a clear indication of any changes in the worm burden. There is a considerable variation between sheep even of the same flock in the number of worms they harbour ; therefore it was decided not to slaughter a small number of animals at frequent intervals. Furthermore, it was desirable that there should be a fair representation of each age group at each slaughtering. It was finally planned to have four main slaughterings, each of 50 sheep, containing representatives of each age group ; each group of fifty was made up of 10 hoggs*, 5 or 6 gimmers*, and 34 or 35 ewes varying in age up to draft ewes*.

The times of slaughter were based on the seasonal fluctuations which occur in the worm egg output of hill sheep. The first group of 50 sheep was slaughtered in the last week of August, at a time when the worm egg output is on the decline and stands roughly between the high spring peak and the low level of January ; the second group was slaughtered in January, when the worm egg output is usually at its lowest ; the third in early April, about a week or so before lambing commences, and at a time when the worm egg output is showing a definite increase ; the fourth in the first week of June, when the worm egg output is at, or about, its peak.

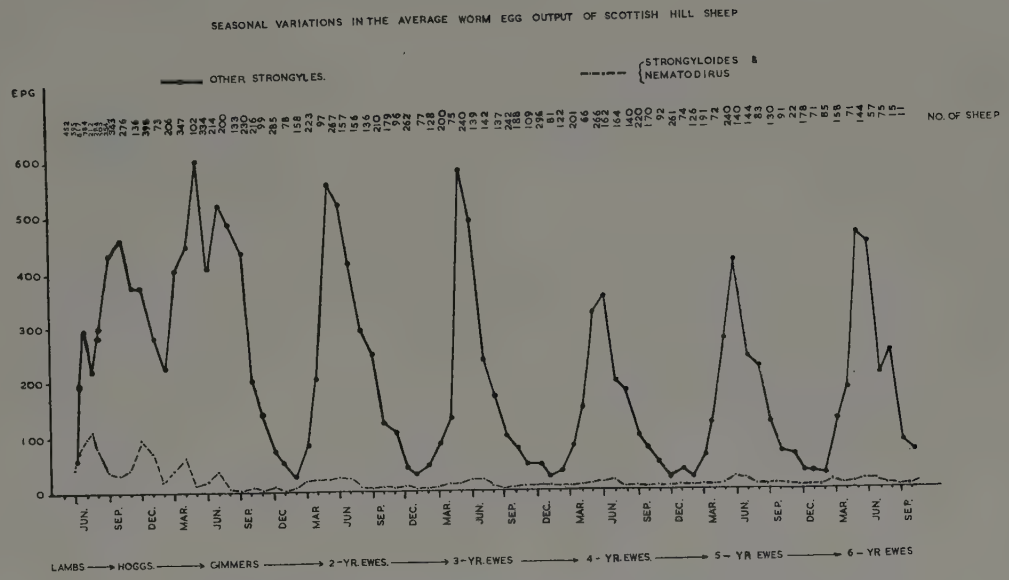
Twenty sheep consisting of representatives of all the age groups were bought in excess of requirements in order to provide against the possibility of a high death rate during the season. Fortunately there were only three deaths during the year and the remainder were therefore available for slaughter ; this was carried out in the second week of July. There were also lambs born to the ewes which still remained after the April slaughtering ; some of these lambs were slaughtered in June and some in July.

The management of the sheep followed that normally adopted on the farm, and, with the exception that there was no dosing against worms, all the usual practices were observed.

It had been realised that after each batch of fifty sheep had been slaughtered there would be a corresponding reduction in the number of sheep on the hill and that the effect of this would be to reduce the contamination with infective larvae below that which would normally occur with a fully populated heft. This, however, was remedied to

*Ewe " hoggs " is the term given to female sheep from weaning in August until they are first sheared in late June or early July, when they become " gimmers " ; at the following shearing they become " ewes " ; on this farm they are sold off the hill as draft ewes at six years old.

GRAPH I.



Graph 1.—Worm egg count of an " average " Scottish hill sheep throughout life ; based on worm egg counts from numerous sheep on many farms from several districts of Scotland.

TABLE I.

Average egg counts of the "Other Strongyles" ("O.S."), of Strongyloides papillosus *("S") and* Nematodirus *spp. ("N") for the sheep which were subsequently slaughtered, and the number of sheep on which this data is based.*

		Aug. 23rd	Oct. 5th	Nov. 11th	Jan. 10th	Feb. 15th	March 15th	April 4th	May 3rd	May 23rd	June 6th	June 27th	July 11th
Lambs.	No. of Sheep	—	—	—	—	—	—	—	—	16	35	19	20
	Average "O.S.".	—	—	—	—	—	—	—	—	9	23	121	240
	Average "S".	—	—	—	—	—	—	—	—	9	6	8	22
	Average "N".	—	—	—	—	—	—	—	—	0	2	8	12
Hoggs.	No. of Sheep.	47	37	37	37	27	27	26	16	16	16	6	6
	Average "O.S.".	150	282	500	386	341	519	794	247	207	91	33	50
	Average "S".	53	34	65	50	39	24	65	22	9	13	8	8
	Average "N".	8	4	5	34	26	15	29	6	6	0	0	0
Gimmers.	No. of Sheep.	23	17	17	17	12	12	12	7	7	6	2	2
	Average "O.S.".	178	179	226	94	63	175	333	229	121	108	275	275
	Average "S".	43	29	12	18	8	29	121	21	14	17	50	0
	Average "N".	0	0	3	0	4	0	4	21	21	0	25	0
Ewes.	No. of Sheep	142	114	114	113	76	78	79	39	44	42	9	9
	Average "O.S.".	190	97	71	55	62	99	173	242	220	159	94	78
	Average "S".	26	13	13	8	20	25	14	22	10	11	22	0
	Average "N".	0	0	1	0	0	0	1	1	5	0	0	0

TABLE II.

Average number of eggs of the "Other Strongyles" ("O.S.") and of Strongyloides papillosus, *and* Nematodirus *spp. together ("S.N.") for all age groups for each month of the year, and the number of sheep on which this data is based.*

		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June 1–15	June 16–30	July 1–15	July 16–31	Aug. 1–15	Aug. 16–31	Sept. 1–15	Sept. 16–30
Lambs.	No. of Sheep.	—	—	—	—	—	—	—	—	452	595	617	784	638	748	505	354
	Average "O.S.".	—	—	—	—	—	—	—	—	54	196	293	264	212	284	292	440
	Average "S.N.".	—	—	—	—	—	—	—	—	38	90	100	111	81	83	46	44
Hoggs.	No. of Sheep.	343	276	136	398	73	206	347	102	334		214		200		133	
	Average "O.S."	444	362	363	273	212	396	437	597	383		517		496		422	
	Average "N.S.".	31	43	99	72	20	48	67	13	20		40		6		10	
Gimmers.	No. of Sheep.	230	216	99	285	78	158	223	97	267		157		156		136	
	Average "O.S.".	197	123	76	45	20	80	194	550	507		386		283		240	
	Average "S.N.".	5	11	3	7	19	22	23	27	23		7		6		8	
2-year Ewes.	No. of Sheep.	210	179	96	262	77	128	200	75	240		139		142		137	
	Average "O.S.".	118	100	34	22	36	78	122	577	474		230		295		97	
	Average "S.N.".	5	8	3	4	6	12	12	19	19		7		3		4	
3-year Ewes.	No. of Sheep.	242	188	109	296	81	122	201	66	266		162		164		140	
	Average "O.S.".	72	39	40	17	26	73	141	317	351		190		174		91	
	Average "S.N.".	6	5	7	4	6	8	10	13	17		3		5		1	
4-year Ewes.	No. of Sheep.	220	170	92	261	74	126	191	72	240		140		144		83	
	Average "O.S.".	62	38	17	28	14	50	124	285	406		224		212		109	
	Average "S.N.".	4	3	4	3	5	4	6	13	11		3		4		6	
5-year Ewes.	No. of Sheep.	59	91	22	178	71	85	158	71	144		57		75		15	
	Average "O.S.".	130	55	23	20	17	103	170	450	431		193		243		77	
	Average "S.N.".	3	1	2	2	11	5	7	14	13		1		2		0	

some extent by the introduction of young sheep on to the hill and, although the number introduced was not equal to the number taken away, the fact that they were young sheep would tend to keep up the number of infective larvae on the ground to something approaching the normal number.

Slaughtering was carried out at the Edinburgh City Abattoir; observations were made on the condition of the carcases and a note was taken of the presence of worms in the lungs, liver and other organs. The abomasa and intestines were then brought back to the laboratory, where the contents of the abomasum, of the small intestine and of the large intestine of each sheep were sedimented separately in buckets. After sedimentation the contents were preserved in weak formalin and stored until a full examination could be made. All the worms belonging to the following species were counted separately : *Haemonchus contortus, Bunostomum trigonocephalum, Oesophagostomum venulosum, Chabertia ovina* and *Trichuris ovis*. The numbers of the smaller and more numerous worms were estimated by a dilution technique. The contents of the abomasum and small intestine of each sheep were diluted separately with water up to 500 cc's., or in cases where the contents were very bulky up to 1,000 cc's., and after thorough mixing 10 cc's. were drawn off and the worms in this quantity were then counted ; this was done by placing successive smears on a glass slide and examining these under the low power of a microscope, until the whole of the 10 cc. sample was completed. This method had the advantage that the magnification was high enough for rapid species identification, for observations to be made on the state of maturity of the females and also for the youngest larval stages to be seen and counted.

Investigations in 1949–50.—Naerland (1949) suggested that the spring increase in worm egg counts in sheep might be the result of adults which had developed in the spring from larvae which had remained dormant in the mucous membrane of the abomasum and small intestine throughout the winter. Kotlan (1949) and others have also drawn attention to the tendency for larvae of certain species of nematodes in various hosts to lie dormant in the mucous membranes and other tissues. It was therefore decided to investigate this possibility in Scottish hill sheep.

The one half of a heft of Cheviot ewes, which had remained after the purchases of 1948, was bought from Mr. Linton of Gilmanscleuch. It was considered that by slaughtering two ewes every fortnight

between December and mid-Summer and by examining the mucous membranes the presence or absence of hibernating larvae could be determined. The abomasum and the first ten feet of the small intestine of each sheep were cut into pieces, placed in a compressorium and examined under a dissecting microscope ; small portions were also sectioned. In addition periodic worm egg counts were made before slaughter, and, using the same technique as in the previous year, estimates were made of the worm burdens.

Results in 1948–49.

That the sheep slaughtered in 1948–49 were in no way abnormal in their worm burden is evident by comparing Table I, which shows their worm egg counts, with Table II and Graph 1, which gives the average worm egg counts of a large number of sheep, both Blackfaces and Cheviots, on many hill farms throughout Scotland. The average worm egg counts of the " Other Strongyles "* of *Nematodirus* spp. and of *Strongyloides papillosus* for the sheep slaughtered in 1948–49 are, of course, based on decreasing numbers of animals. In Table II the egg counts of *Nematodirus* and *Strongyloides* have been added together, since neither species is important except in lambs and hoggs. In lambs in particular, the incidence of these species varies considerably from farm to farm.

Graphs 2 to 12 show the average number of worms of the various species of nematodes which were most commonly found in these sheep at each slaughtering. These graphs also include the worms from the fifteen and twenty lambs, which were slaughtered in June and July respectively. At the July slaughtering only six hoggs, two gimmers and nine ewes were available.

Worms of the Abomasum.

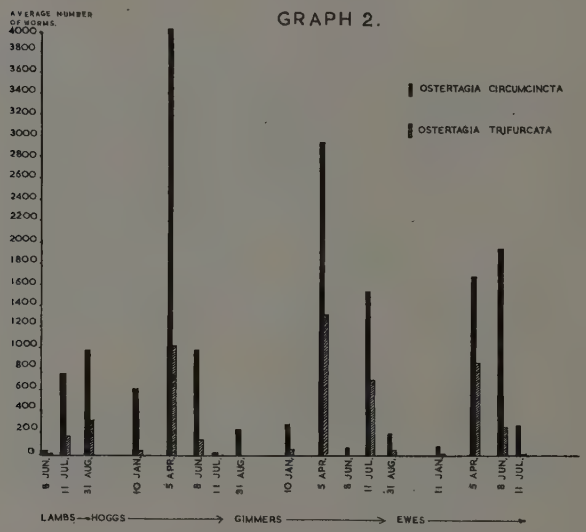
Ostertagia spp.

Graph 2 shows the average number of *Ostertagia circumcincta* and *O. trifurcata*. It has been assumed that the females of the two species were in the same ratio to each other as were the males.

Lambs.—These species were the first to appear in the lambs in appreciable numbers ; by mid-July they had increased to an average of just under 1,000 adults per lamb and by the end of August there had been a further slight increase. *Ostertagia* spp. were found in 12 of the 15 lambs in June, and in all the lambs in July.

* In this paper " Other Strongyles " include all the Strongyle eggs found in these sheep, with the exception of those of *Nematodirus* spp.

Hoggs.—Although there was a fall in numbers, between August and January it was not very marked, but by the beginning of April the average number of mature *Ostertagia* spp. (5,100) had increased eight-fold over the January counts, and the lowest numbers found were 650 *O. circumcincta* and 200 *O. trifurcata*. By early June there had again been a very marked reduction in numbers.



Graph 2.—Average number of *Ostertagia circumcincta* and *O. trifurcata* of the different age groups at each slaughtering.

Gimmers.—*Ostertagia* spp. in gimmers increased twelve-fold between January and April but, as the numbers in the gimmers in January were less than in the hoggs, this only involved an increase to an average of 4,300 adults of the two species. After April the numbers again fell.

Ewes.—In the ewes the increase by April was more than twenty-fold, and this was maintained until June. Again the increase was more marked in the ewes because the January totals were lower than those of the younger sheep. Eighteen were apparently free of *Ostertagia* spp. in January, but none in April and only three in June.

Table III shows the maximum infestations with *Ostertagia* spp. in each age group at each slaughtering.

Haemonchus contortus.

The average number of *H. contortus* found in all age groups at each slaughtering is shown in Graph 3.

Lambs.—One female worm only was found in one of the lambs slaughtered in June. On 11th July fourteen of the twenty lambs were still free of infestation and seven was the greatest number found.

TABLE III.

Greatest number in any one sheep of adult Ostertagia circumcincta (" *O.c.*") *and* Ostertagia trifurcata (" *O.t.*") *in each age group at each slaughtering.*

		31st August	10th January	5th April	8th June	11th July
		Max.	Max.	Max.	Max.	Max.
Lambs ..	O.c.	—	—	—	200	2,300
	O.t.	—	—	—	50	500
Hoggs ..	O.c.	3,000	2,500	8,900	3,150	50
	O.t.	850	350	2,650	750	0
Gimmers ..	O.c.	800	800	5,400	250	2,900*
	O.t.	0	250	2,750	0	1,250*
Ewes ..	O.c.	1,750	750	6,700	12,350	650
	O.t.	600	150	3,350	1,900	150

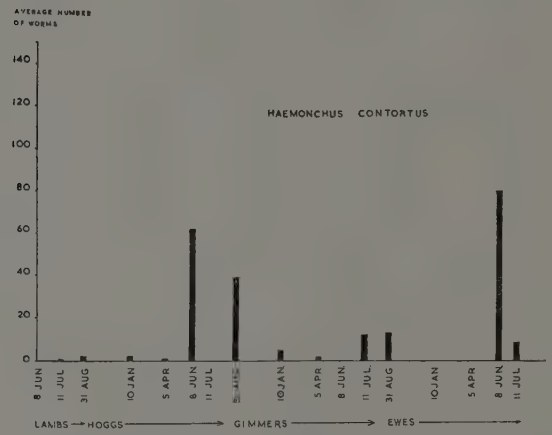
* Based on only two gimmers.

Hoggs.—In hoggs, only at the June slaughtering were there any appreciable numbers of *H. contortus*; two had comparatively heavy infestations (340 and 250 adults) and one had a lighter infestation—31 adults; the other seven hoggs were free of this worm. In July all five were free.

Gimmers.—In August four of the gimmers were free of *H. contortus* but the other two carried 127 and 108 adults. At the other slaughterings the gimmers were free or carried only very light infestations.

Ewes.—In August twenty-nine of the ewes carried no *H. contortus*, three carried very light infestations, but in two of the ewes there were 227 and 192. In January none of the ewes was infested and in April only three carried very light infestations. In June however, while twenty carried no *H. contortus*, six had light infestations, three carried between 80 and 90 worms, and another six harboured between 232 and 674 adults.

GRAPH 3



Graph 3.—Average numbers of *Haemonchus contortus* in the different age groups at each slaughtering.

Trichostrongylus axei.

Graph 4 illustrates the average number of *T. axei* found.

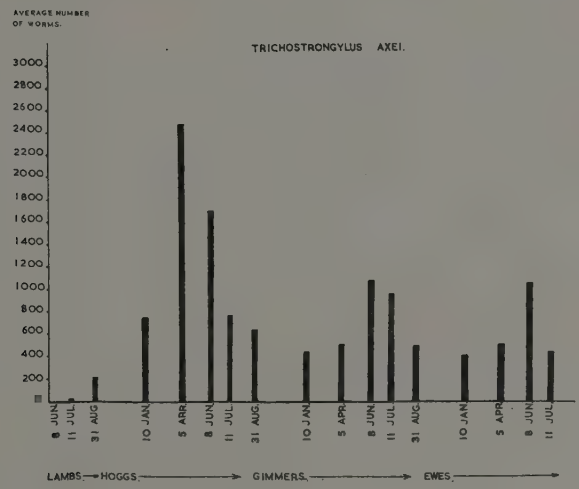
Lambs.—None was recovered from the lambs slaughtered on 8th June, and five weeks later there was an average of only just over twenty per lamb.

Hoggs.—At the end of August the average was just over two hundred, and this species, unlike the other stomach worms, did not show a fall but an increase in numbers at the January slaughtering. In January there were 3,500 in one hogg, but the others carried 700 or less ; in April only one carried under 700. At the January, April and June slaughterings some *T. axei* were found in all the hoggs.

Gimmers.—Most gimmers carried only a few hundred *T. axei* until late spring when the infestations became rather heavier.

Ewes.—In general the seasonal pattern of infestation was similar to that in the gimmers, and as in the hoggs the infestations did not reach very low levels in January, but did not rise appreciably until late spring and early summer.

GRAPH 4.



Graph 4.—Average numbers of *Trichostrongylus axei* in the different age groups at each slaughtering.

The maximum infestation with *Trichostrongylus axei* in each age group at each slaughtering is included in Table IV, which also gives the same data for *T. colubriformis* and *T. vitrinus*. It has been assumed that the females of the two latter species were in the same ratio as the males.

Worms of the Small Intestine.

Trichostrongylus spp.

Graph 5 shows the average number of *T. vitrinus* and *T. colubriformis* found in the different age groups.

Lambs.—Early in June three lambs had light infestations and at the slaughtering five weeks later ten lambs were lightly infested.

Hoggs.—In August the incidence had increased, no hogg was free of both species, and by January the infestations were slightly heavier. In April all the hoggs were infested, and there was a three-fold increase in the average infestation of *T. vitrinus* over the January average. *T. colubriformis* was less numerous but showed a spring increase. By June, although all the hoggs were infested, the infestations were lower.

TABLE IV.

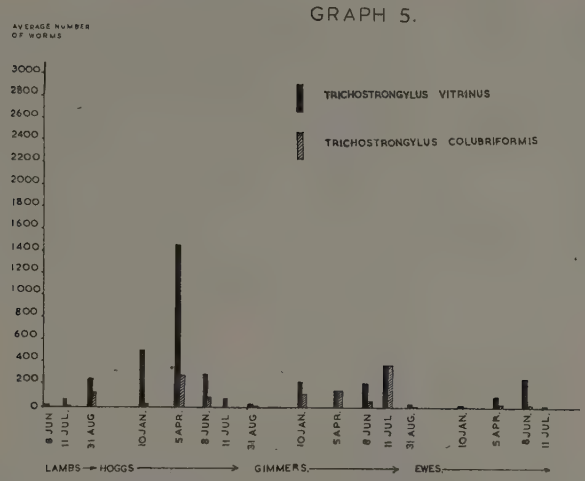
Greatest number of adult Trichostrongylus axei (" *T.a.*"), T. vitrinus (" *T.v.*") *and* T. colubriformis (" *T.c.*") *in each age group at each slaughtering.*

		31st August	10th January	5th April	8th June	11th July
		Max.	Max.	Max.	Max.	Max.
Lambs ..	T.a.	—	—	—	0	150
	T.v.	—	—	—	200	150
	T.c.	—	—	—	0	0
Hoggs ..	T.a.	650	3,500	5,550	3,000	1,500
	T.v.	800	2,200	3,200	750	250
	T.c.	700	200	1,350	350	0
Gimmers ..	T.a.	1,800	900	1,100	2,400	1,800*
	T.v.	150	500	400	1,000	750
	T.c.	50	250	700	250	750
Ewes ..	T.a.	2,850	3,800	2,400	4,750	1,700
	T.v.	500	100	950	4,500	50
	T.c.	100	100	300	450	0

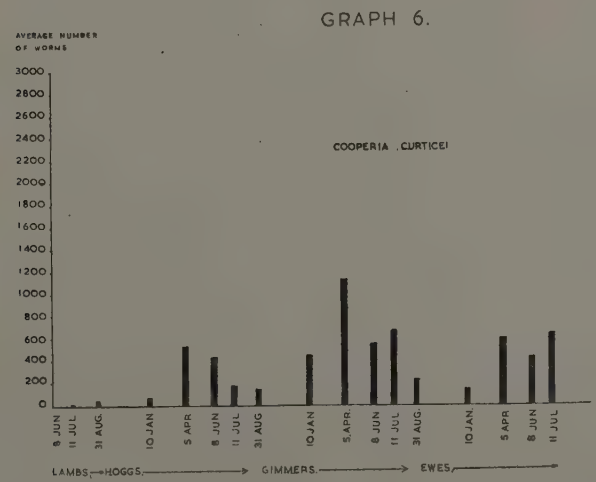
* Based on only two gimmers.

Gimmers and Ewes.—The level of the infestations with both *T. vitrinus* and *T. colubriformis* in most of the gimmers and ewes was low.

The ratio of *T. vitrinus* to *T. colubriformis* was slightly under 5 to 1.



Graph 5.—Average numbers of *Trichostrongylus vitrinus* and *T. colubriformis* in the various age groups at each slaughtering.

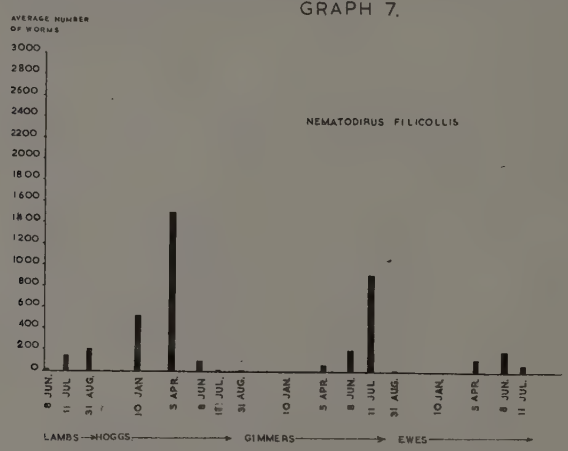


Graph 6.—Average numbers of *Cooperia curticei* in the different age groups at each slaughtering.

Cooperia curticei.

Graph 6 shows the average number of *C. curticei* carried by the slaughtered sheep.

Lambs.—None of the lambs slaughtered in June and only one of those slaughtered in July was infested, and it carried only a few adults.



Graph 7.—Average numbers of *Nematodirus filicollis* in the different age groups at the five slaughterings.

Hoggs.—In August and January the level of infestation in the hoggs with *C. curticei* was still low, but by April there had been a marked increase, although the infestations did not reach high levels.

At the June slaughtering all the hoggs carried some *C. curticei.*

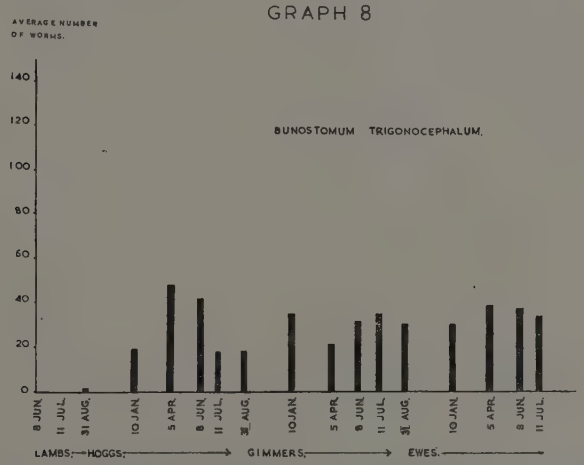
Gimmers and Ewes.—*C. curticei*, after showing a definite increase in April, tended to persist into the summer and on an average the infestations were higher than in the hoggs. The heaviest infestation amounting to 8,800 was found in a gimmer in April.

Nematodirus filicollis.

Although *N. spathiger* has been recorded in sheep in Scotland, only *N. filicollis* was identified during this investigation. In Ettrick, judging by worm egg counts in lambs, the infestations are not as heavy as in some other districts of Scotland, particularly on some of the hill farms nearer the Border. Graph 7 shows the average number of *N. filicollis* recovered from these sheep.

Lambs.—At the slaughtering early in June seven of the fifteen lambs were apparently still free, but five weeks later only two out of twenty lambs were uninfested ; 400 worms was the highest number found.

Hoggs.—At the end of August three out of ten hoggs were uninfested, and the heaviest infestation was 750, but both in January and April none of the hoggs was free of *Nematodirus* ; the heaviest infestations were 1,750 and 2,100 respectively ; by June six out of ten hoggs carried no adults and the heaviest infestations had dropped to 600. Between August and January, and between January and April there were almost three-fold average increases.



Graph 8.—Average numbers of *Bunostomum trigonocephalum* in the different age groups at each slaughtering.

Gimmers and Ewes.—In general infestations with *N. filicollis* were very low in gimmers and ewes throughout the year, although there was some spring increase in numbers ; however, in July one of the two gimmers slaughtered carried 1,800 adults.

Bunostomum trigonocephalum.

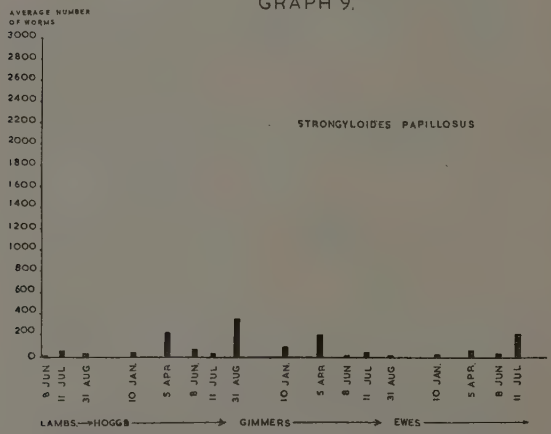
B. trigonocephalum is one of the common species in Scottish hill sheep. Graph 8 shows that there is no marked seasonal difference in the average numbers of this species.

Lambs.—Early in June none was found in any of the lambs; in July one adult was found in two of the twenty lambs slaughtered.

Hoggs and Gimmers.—At the end of August six of the ten hoggs were still free of *B. trigonocephalum*, but subsequently no hogg or gimmer was free of this hookworm.

Ewes.—At the August, June and July slaughterings only one ewe and at the January and April slaughterings only two ewes were free of *B. trigonocephalum*. The infestations in the ewes varied from 4 to 145 in August, from 1 to 230 in January, from 4 to 202 in April, from 3 to 161 in June and from 2 to 78 in July.

GRAPH 9.



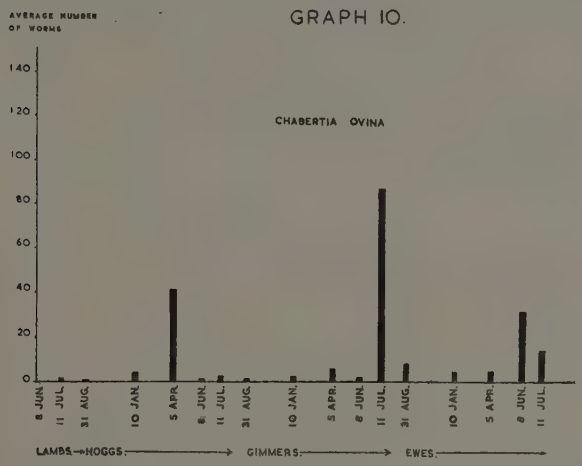
Graph 9.—Average numbers of *Strongyloides papillosus* in the different age groups at the five slaughterings.

Strongyloides papillosus.

The average infestations of *S. papillosus* are shown in Graph 9. In sheep on the hill it is seldom an important species, but in Scottish Blackface rams, which are kept for the greater part of the year in enclosed paddocks or sheds, very heavy infestations have been found.

Lambs.—The eggs of *S. papillosus* are among the first, if not the first, to appear in the faeces of Scottish hill lambs. This species was not found in eight of the fifteen lambs slaughtered in June, nor in twelve of the twenty slaughtered in July; in June 100 females and in July 650 females were the heaviest infestations found.

Hoggs.—At both the August and January slaughterings no females of this species were found in six of the ten hoggs and the other hoggs carried only light infestations. In April and June none was found in two of the ten hoggs ; at the former slaughtering 800 and at the latter 250 were the heaviest infestations.



Graph 10.—Average numbers of *Chabertia ovina* in the various age groups at the five slaughterings.

Gimmers and Ewes.—Infestations with *S. papillosus* were also low in gimmers and ewes and many were free or almost free ; however, the incidence was highest in the spring, but the highest infestations amounted to only a few hundred females.

Capillaria longipes.

C. longipes is of no importance in Scottish hill sheep.

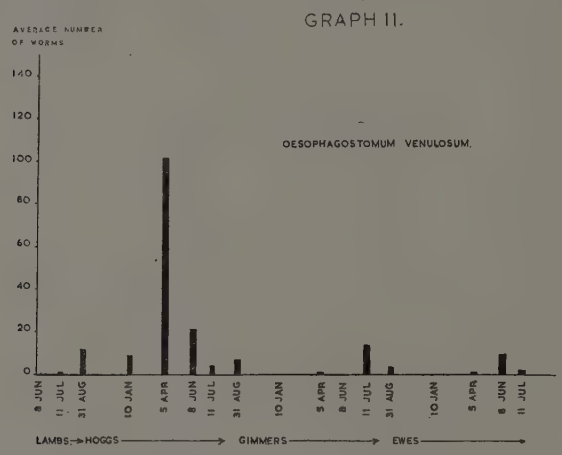
Lambs and Hoggs.—No *C. longipes* was found in the lambs and none in the hoggs until they were over a year old.

Gimmers and Ewes.—In only a few of the gimmers and ewes was this worm found. It seemed to be slightly more common in the sheep slaughtered during the summer.

Moniezia spp.

Lambs.—*Moniezia* spp. were found in ten of the fifteen lambs slaughtered on 8th June, and in all, except one, of the twenty lambs slaughtered five weeks later, when the infestations were also heavier.

Hoggs.—At the end of August tapeworms were seen in only six of the ten hoggs slaughtered. In January it was present in only two of the hoggs, but by April nine out of the ten harboured the parasite; however, by June nine of the ten hoggs were free and all were free in July.



Graph 11.—Average numbers of *Oesophagostomum venulosum* in the different age groups at each slaughtering.

Gimmers and Ewes.—Approximately forty per cent. of the gimmers and ewes harboured *Moniezia* spp.; the incidence varied from seventeen per cent. in August to sixty per cent. in April. Heavy infestations were found in two of the ewes in April and two in June.

Worms of the Large Intestine.

Chabertia ovina.

Graph 10 shows the average infestations with *C. ovina*, and Table V shows the maximum numbers of this and the other species found in the large intestine.

Lambs.—Only one *C. ovina* was found in the lambs in June; in July nine lambs were infested.

Hoggs.—Not until these sheep were nearly a year old, i.e., in April, were heavy infestations of *C. ovina* found, when three out of ten hoggs had infestations of over eighty adults. But at the subsequent killings very few *C. ovina* were found.

TABLE V.

Greatest number of adult Chabertia ovina (" *C.o.*"), Oesophagostomum venulosum (" *O.v.*") *and* Trichuris ovis (" *T.o.*"), *in each age group at each slaughtering.*

		31st August	10th January	5th April	8th June	11th July
		Max.	Max.	Max.	Max.	Max.
Lambs ..	C.o.	—	—	—	1	11
	O.v.	—	—	—	0	5
	T.o.	—	—	—	4	90
Hoggs ..	C.o.	4	11	103	4	3
	O.v.	27	13	300	87	22
	T.o.	38	12	8	13	4
Gimmers ..	C.o.	3	5	16	9	166*
	O.v.	24	2	5	0	27
	T.o.	0	1	7	0	5
Ewes ..	C.o.	66	32	37	276	70
	O.v.	64	3	13	169	14
	T.o.	2	3	2	1	1

* Based on only two gimmers.

Gimmers.—Only a few *C. ovina* were found in the gimmers, with the exception of one killed in July which had a heavy infestation ; this accounts for the highest column in Graph 10.

Ewes.—Only in a few ewes were heavy infestations found ; these occurred mostly in June.

Oesophagostomum venulosum.

The average infestations with *O. venulosum* are shown in Graph 11 ; Table V also contains data on this species.

Lambs.—None was found in the lambs slaughtered in June, but at the July slaughtering eleven out of twenty lambs harboured a few adults.

Hoggs.—Only one hogg in January, three hoggs in June and three hoggs in July were without *O. venulosum*. In April all the hoggs were infested and the average level of the infestations was appreciably higher.

Gimmers and Ewes.—In gimmers and ewes infestations were generally low, and a high proportion of the ewes was free of this worm.

Trichuris ovis.

The maximum numbers of *T. ovis* found in the different age groups at the five slaughterings are shown in Table V.

Lambs.—Even by the 7th June five of the fifteen lambs carried light infestations, and by the 12th July all the lambs were infested; the average infestation was twenty-two adult *T. ovis*.

Hoggs.—At the end of August two of the ten hoggs were free of whipworms; the average infestation was fourteen worms. All the hoggs at the January and April slaughterings were infested, but the average infestation fell to six and four adults respectively.

Gimmers and Ewes.—The incidence and degree of infestation in gimmers and ewes were at very low levels.

Young Stages in the Abomasum and Small Intestine.

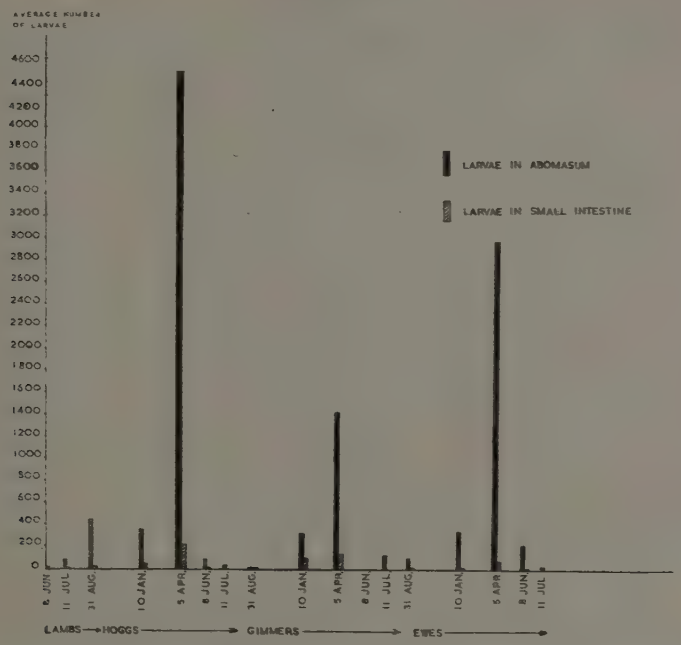
Graph 12 shows the average numbers of fourth and fifth stages of the nematodes found in the lumen of the abomasa and small intestines of the various age groups of the sheep at the five slaughterings, and Table VI shows the highest numbers found. These show that the larvae were very much more numerous in the abomasa than in the small intestines, and some of those found in the small intestine may have been larvae which had been expelled from the abomasum.

Lambs.—When the lambs were slaughtered on 8th June, larvae were found in the contents of the abomasa of two lambs. At the slaughtering five weeks later larvae were found in the abomasal contents of ten, i.e., half, of the lambs; only in one lamb were larvae found in the small intestine.

Hoggs.—At the end of August and in January no larva was found in the contents of the abomasum of a few of the hoggs, but on 5th April larvae were found, often in large numbers, in the contents of the abomasa of all the hoggs; nine weeks later the numbers had fallen and larvae were found in only seven hoggs. The incidence of larvae

in the small intestine was not as high as in the abomasum, but again was highest in April.

GRAPH 12



Graph 12.—Average numbers of fourth and fifth stage larvae in the lumen of the abomasa and small intestines of the various age groups at the five slaughterings.

Gimmers.—At the end of August a very few larvae were found in the contents of the abomasum of one gimmer, but both in January and in April all the gimmers harboured larvae in the abomasa, and at the latter date the numbers were very large. On 8th June, however, none was found. Again the incidence of larvae in the small intestine was highest in January and April.

Ewes.—The numbers of ewes in which larvae were found in the abomasal contents at the four main slaughterings was eight in August, twenty-three in January, thirty-five in April and fourteen in June. As shown in Table VI some very heavy infestations were seen in April.

The corresponding figures for the larvae in the small intestines were three in August and January, nine in April and one in June.

Lung Worms.

Dictyocaulus filaria.

Lambs.—No *D. filaria* was found in any of the lambs slaughtered on 8th June, and in only one of the twenty lambs slaughtered five weeks later.

Hoggs.—This lungworm was found in one hogg slaughtered in January, in three hoggs in April and two hoggs in June.

TABLE VI.

Highest numbers of fourth and fifth stages of nematodes found in the lumen of the abomasa ("A.") and in the lumen of the small intestines ("S.I.") of the four age groups at the five slaughterings.

		31st August	10th January	5th April	8th June	11th July
		Max.	Max.	Max.	Max.	Max.
Lambs ..	A.	—	—	—	50	250
	S.I.	—	—	—	0	50
Hoggs ..	A.	1,350	1,100	12,050	200	150
	S.I.	100	150	900	50	0
Gimmers ..	A.	50	600	3,850	0	250
	S.I.	50	550	350	0	0
Ewes ..	A.	1,350	2,750	18,450	3,750	50
	S.I.	200	50	1,300	50	0

Gimmers.—*D. filaria* was found in two of the gimmers slaughtered in April and one slaughtered in July.

Ewes.—Most of the infestations in the ewes were light ; infestations were found in four in August, three in January, nine in April and two in June ; one of the April infestations was heavy.

Muellerius capillaris.

Lambs.—*M. capillaris* was seen in six of the fifteen lambs slaughtered on 7th June ; five weeks later all the lambs were infested.

Hoggs, Gimmers and Ewes.—All the hoggs, gimmers and ewes were infested, and in some cases the infestations were heavy.

TABLE VII.

Average numbers of the various species of intestinal nematodes which were found in each age group at the five slaughterings.

Date.	Age Group.	No. of Sheep.	*H. contortus.*	*O. circumcincta.*	*O. trifurcata.*	*T. axei.*	Young stages in Abomasum.	*T. colubriformis.*	*T. vitrinus.*	*C. curticei.*	*B. trigonocephalum.*	*N. filicollis.*	*S. papillosus.*	Young stages in Small Intestine.	*O. venulosum.*	*C. ovina.*	*T. ovis.*
31st Aug.	Hoggs.	10	4	990	320	205	460	125	240	65	3	205	40	15	11	1	14
	Gimmers.	6	39	242	0	633	8	8	25	137	34	17	12	0	4	5	1
	Young ewes.	8	1	69	6	244	6	0	25	137	34	17	12	0	4	5	1
	3-year ewes.	7	0	43	0	664	207	0	7	93	49	0	0	7	1	3	0
	4-year ewes.	6	0	325	67	800	117	17	0	692	26	0	17	67	13	12	0
	5-year ewes.	4	0	62	112	137	37	0	0	13	10	0	0	0	1	4	0
	6-year ewes.	9	48	400	72	439	72	0	71	100	22	21	71	0	2	15	0
11th Jan.	Hoggs.	10	2	615	35	740	285	20	505	170	24	520	45	60	9	4	6
	Gimmers.	5	5	300	60	450	330	110	210	450	35	0	100	190	0	2	0
	Young ewes.	8	0	38	25	825	294	13	19	94	38	0	6	6	1	3	0
	3-year ewes.	7	0	121	36	264	614	0	21	86	23	0	14	0	0	2	0
	4-year ewes.	6	0	217	42	425	425	0	8	92	20	0	83	0	0	7	0
	5-year ewes.	5	0	10	0	70	290	10	30	520	32	0	50	10	0	7	0
	6-year ewes.	9	0	56	11	300	222	6	11	50	29	0	28	6	0	2	0
5th Apr.	Hoggs.	10	1	4,055	1,040	2,470	4,470	270	1,470	545	48	1,505	220	225	102	42	4
	Gimmers.	5	2	2,970	1,340	490	1,430	140	140	1,120	21	60	210	130	1	6	1
	Young ewes.	8	0	1,450	638	744	4,188	0	69	438	33	181	119	62	0	1	0
	3-year ewes.	7	2	2,136	1,193	443	4,529	43	136	429	23	0	21	71	0	7	0
	4-year ewes.	6	0	1,358	500	292	1,183	0	0	1,083	28	25	133	8	0	2	0
	5-year ewes.	5	0	2,320	1,510	160	2,780	20	200	340	55	0	0	20	3	13	0
	6-year ewes.	9	0	1,533	806	617	2,044	22	67	706	51	228	78	167	0	2	0
7th June.	Lambs.	15	0	37	3	0	7	0	20	0	0	20	30	0	0	0	1
	Hoggs.	10	62	990	145	1,700	80	90	285	430	42	90	75	10	22	1	2
	Gimmers.	5	0	70	0	1,080	0	50	200	550	31	200	30	0	0	2	0
	Young ewes.	8	87	1,375	275	1,444	94	0	44	388	37	156	69	0	1	11	0
	4-year ewes.	8	14	881	63	644	181	6	125	263	47	206	19	6	0	19	0
	5-year ewes.	6	59	1,558	242	1,042	58	0	8	392	35	17	142	0	8	49	0
	6-year ewes.	5	187	6,660	730	650	880	0	50	750	25	90	0	0	56	101	0
	7-year ewes.	8	86	1,000	131	1,369	50	75	869	488	36	350	19	0	0	5	0
12th July.	Lambs.	20	1	757	167	22	70	2	60	2	0	142	62	2	1	2	22
	Clipped Hoggs.	6	0	17	0	758	33	0	67	175	17	17	42	0	4	2	1
	Gimmers.	2	12	1,550	700	975	125	375	375	650	34	900	50	0	14	88	3
	Young ewes.	2	39	225	0	1,200	0	0	25	425	29	0	175	0	2	26	0
	4-year ewes.	2	0	125	0	475	25	0	0	275	9	0	0	0	1	1	0
	5-year ewes.	2	0	350	75	150	50	0	0	1,625	46	25	150	0	0	1	0
	6-year ewes.	1	0	1,150	0	150	0	0	0	200	63	150	50	0	14	70	0
	7-year ewes.	2	2	0	0	50	0	0	0	375	33	75	125	0	0	1	1

Liver Fluke.

On this farm *Fasciola hepatica* is only rarely found and the few infestations which have occurred have been light, and have apparently not affected these sheep.

Cysticercus tenuicollis.

Many cysts of *C. tenuicollis* were found, but the number in individual sheep was small.

Summary 1948–49.

Table VII shows the average number of each species of intestinal nematodes in each age group at each slaughtering.

Results in 1949–50.

In 1949–50 two ewes were slaughtered in mid-December, and subsequently two ewes were slaughtered at fortnightly intervals from mid-January until the end of June ; this made fourteen slaughterings and involved a total of twenty-eight ewes. The main object was to determine whether or not larvae hibernate in the intestinal mucosa.

Larvae in the Abomasum and Small Intestine.

In the mucosa.—The investigation showed that there was no evidence that the larvae hibernated in the mucosa of the abomasum or of the small intestine. In the abomasum only thirteen and one larvae were found on the 22nd March and one on the 3rd May ; in the small intestine one larva was found on the 22nd February. No larva was seen in the mucosa of the abomasum and small intestine at any other time in the slaughtered ewes.

In the lumen.—In contrast the lumen of the abomasum harboured large numbers of larvae, particularly in the early spring ; this is shown in Table VIII.

The number of larvae found in the lumen of the small intestines was never more than about fifty.

The relatively small number of larvae found in the two ewes slaughtered on 3rd May may be due to the fact that they had lost their lambs in the first half of April, one a single and the other triplets.

Worms of the Abomasum.

No *Haemonchus contortus* was found until 31st May, when there were 24 in one ewe ; subsequently 71 and 2 were found in the ewes slaughtered on 28th June.

TABLE VIII.

Number of larvae in the lumen of the abomasa of the twenty-eight ewes at the fourteen slaughterings.

Dec.	January		February		March		April		May			June	
14th	11th	25th	8th	22nd	8th	22nd	5th	19th	3rd	17th	31st	14th	28th
0	0	50	0	350	550	5,850	5,300	5,200	0	400	150	0	0
0	250	50	0	400	600	22,300	10,000	5,850	150	600	4,200	50	0

TABLE IX.

Numbers of Ostertagia circumcincta, *of* O. trifurcata *and of* Trichostrongylus axei *in each of the twenty-eight ewes.*

	Dec.	January		February		March		April		May			June	
	14th	11th	25th	8th	22nd	8th	22nd	5th	19th	3rd	17th	31st	14th	28th
O. circumcincta	0	50	0	0	0	0	350	350	150	0	0	350	0	250
	0	50	50	0	0	100	400	750	1,300	0	350	8,500	0	1,750
O. trifurcata	0	0	0	0	0	0	50	0	0	0	0	0	0	0
	0	0	0	0	0	100	150	150	150	0	0	850	0	300
T. axei	0	0	0	0	0	0	50	0	150	0	300	200	50	0
	350	750	300	0	500	300	1,300	350	650	900	2,600	550	900	1,300

The numbers of adult *Ostertagia* spp. and *Trichostrongylus axei* found in each of the ewes are given in Table IX.

The numbers of *Ostertagia circumcincta* increased in most ewes in the spring; but the increase in *O. trifurcata* was less marked; the ratio of these two species was about 8 : 1.

The numbers of *Trichostrongylus axei* also increased in the spring, but the numbers harboured during the winter in about half of the ewes were greater than the numbers of *Ostertagia* spp.

Worms of the Small Intestine.

Few *Trichostrongylus* spp. were found. On 25th January one ewe harboured 50 *T. colubriformis*; on 5th April another ewe carried 50 *T. vitrinus* and on 31st May 600 *T. vitrinus* were found in one ewe.

The numbers of *Cooperia curticei* and *Bunostomum trigonocephalum* which were in these ewes are shown in Table X.

Worms of the Large Intestine.

Table XI shows the numbers of *Oesophagostomum venulosum* and *Chabertia ovina* in these ewes.

Summary 1949–50.

Although the numbers of ewes at each slaughtering in 1949–50 were small the worm counts confirm the results of 1948–49, which showed that the increase in worm egg counts in the spring is correlated with an increase in the worm burden following an intake of large numbers of larvae.

Body Weights.

On Scottish hills sheep lose weight during the winter and this may be an important factor contributing to the high helminth burdens in the spring. Table XII shows the average body weights of ewes, gimmers and hoggs on a hill farm on the Pentland Hills in 1946–47, and of hoggs on the same farm in 1947–48. In 1946–47 the maximum numbers weighed were 52 hoggs, 48 gimmers, and 102 ewes; in 1947–48 55 hoggs were weighed.

It should be noted that the ewes and gimmers lambed between the April and June weighings.

In 1946–47, between November and April the average loss of weight was 7.2% in the 4-year old ewes, 9.8% in the 3-year old ewes, 9.8% in the 2-year old ewes, 11.4% in the gimmers and 18.6% in the hoggs and 13.2% in the hoggs in 1947–48.

TABLE X.

Numbers of Cooperia curticei *and* Bunostomum trigonocephalum *found in each of the twenty-eight ewes*

	Dec.	January		February		March		April		May			June	
	14th	11th	25th	8th	22nd	8th	22nd	5th	19th	3rd	17th	31st	14th	28th
C. curticei	0	0	0	0	0	50	100	0	0	50	0	0	0	0
	0	250	50	50	350	1,050	200	100	1,100	150	150	300	50	200
B. trigonocephalum ..	2	24	15	9	0	12	12	7	1	4	0	9	8	8
	7	32	41	61	5	40	32	19	15	49	20	30	10	39

TABLE XI.

Numbers of O. venulosum *and* C. ovina *in each of the twenty-eight ewes.*

	Dec.	January		February		March		April		May			June	
	14th	11th	25th	8th	22nd	8th	22nd	5th	19th	3rd	17th	31st	14th	28th
O. venulosum	0	0	0	0	0	0	0	0	0	0	2	0	0	5
	0	0	0	0	0	0	0	0	0	33	29	0	0	55
C. ovina	0	2	0	1	0	0	0	0	0	0	4	0	0	80
	1	8	0	3	0	1	0	0	7	187	106	2	0	210

During the severe winter of 1946–47 hay was fed to the sheep on this hill. Orr and Fraser (1932) showed even greater loss in weight in ewes in Argyllshire during the winter.

Discussion.

The results show very clearly that the seasonal variation in the worm burden of hill sheep follows the same general pattern as that obtained in previous investigations on the numbers of nematode eggs passed in the faeces, namely a marked increase in the spring months. This increase occurs in several of the species and in particular in those which are most common and are usually found in greatest numbers. In the main the genera *Ostertagia* and *Trichostrongylus* contributed most to the spring increase and they are the genera most commonly associated with outbreaks of helminthiasis in young sheep in the early months of the year in Scotland. Another feature of the worm burden in March and April was the large number of young stages found, particularly in the abomasum. This would seem to indicate that the sheep were not only picking up large numbers of larvae at that time, but that these larvae were able to establish themselves in the host and produce heavy infestations ; both fourth and fifth stages were present in abundance and usually the number far exceeding that of adult forms. It may be concluded, therefore, that although some development may take place in the larvae ingested from the pasture they do not all reach maturity even in the spring. Counts of over 20,000 larvae have been obtained in the abomasum, but it is rare for a hill sheep to harbour infestations of that magnitude, except in cases of clinical helminthiasis.

The increase in the worm burden in the spring would appear to be sufficient to account for the increase in worm egg output at that time and there is therefore no need to postulate an increase in the egg laying capacity of the worms. Since our investigations show no evidence that the larvae remain dormant in the mucosa throughout the winter, it is clear that heavy infestations can be acquired when the temperatures on hill pastures would appear to be too low for the rapid development of the free-living stages. *Ostertagia* spp. and *Trichostrongylus* spp. appear very early in the year and *Ostertagia* spp. in particular probably contribute more than any other worms to the spring increase in worm egg output. There is evidence that *Ostertagia circumcincta* is adapted to low temperatures. Thus Ross and Gordon (1936), quoting unpublished observations made by Kauzal in Australia, state that the eggs of *O. circumcincta* " may develop and hatch at temperatures never

TABLE XII.

Average weights in pounds of the ewes, gimmers and hoggs of a small flock of Blackface sheep.

1946–47			Nov. 18th	Dec. 17th	Jan. 17th	March 12th	April 4th	June 2nd	Fleece Weight	July 9th	August 19th
Ewes	—	—	118.8	116.7	116.9	115.9	108.9	106.3	5.8	111.8	112.3
Gimmers	—	—	111.3	109.0	107.7	98.9	98.6	99.4	6.0	105.6	111.7
Hoggs	—	—	78.9	71.2	74.1	67.8	64.2	83.8	6.3	92.3	98.6
1947–48	August 19th	Oct. 7th	Nov. 11th		Jan. 14th		April 5th	June 15th			August 27th
Hoggs	61.1	65.2	72.8	—	64.1	—	63.2	80.0	5.1	—	94.6

rising above 5°C (41°F)" and "its prevalence in cold highlands and in the winter rainfall areas in part depend on this."

Examinations of grass samples throughout the winter of 1947 from exposed situations on Scottish hills and also on fields (Harbour, Morgan, Sloan and Rayski, 1946) have shown the presence of live infective larvae. It may therefore be concluded that there are sufficient larvae on Scottish hill pastures to provide heavy infestations at any time of the year and that the level of the infestation is influenced more by the host's resistance than by the rate of intake of infective larvae. This resistance may be particularly low in the spring after a winter of exposure to adverse weather and to a low level of nutrition. Indeed as already shown there is a very marked fall in the weight of hill sheep between November and April and to this, in the case of breeding sheep, must be added the strain of carrying a lamb. During these investigations it has been found that there is a tendency for the spring increase in worm egg output to occur earliest in the first ewes to lamb, and to be less marked in non-pregnant ewes.

This investigation has shown that *Ostertagia* spp. are the main contributors to the spring increase in the worm burden and probably, therefore, to the increase in worm egg output. Furthermore, although the younger stages were not identified it was considered that the large numbers found in late winter and early spring consisted mainly of *Ostertagia* spp.

In general it has been held that *Haemonchus contortus* is of less importance in Scottish sheep than *O. circumcincta*, and our observations support this view, although some exceptionally heavy infestations with *H. contortus* have been seen in hill ewes in some districts. The higher temperature requirements for development and survival of the free-living stages of *H. contortus* would tend to make it less well adapted to Scottish conditions than *Ostertagia* spp. and some of the other trichostrongyles. *H. contortus* was virtually absent in April, when heavy infestations of *Ostertagia* were already established, but by June although many were still free some of the ewes had appreciable infestations of *H. contortus*. *Ostertagia* spp. on the other hand had already begun to decline in numbers in the young sheep although many of the ewes still carried heavy infestations. It is probable therefore that the first increase in egg output in the spring is mainly due to *Ostertagia* and *Trichostrongylus* spp., but by May and early June *H. contortus*, a prolific egg layer, will be making some contribution to the egg production which is usually at its peak about that time.

Although there is a marked spring increase in the numbers of *Trichostrongylus axei*, this species does not fall in numbers in January to the extent shown by *Ostertagia* spp.; it also tends to persist in appreciable numbers throughout the life of hill sheep. The other *Trichostrongylus* spp. are less numerous, but in hoggs in the spring *T. vitrinus* may be an important parasite. *T. colubriformis* is practically always less numerous in Scottish hill sheep.

Cooperia curticei increases in numbers in the spring and tends to persist through the summer; it also is a species which is as numerous in mature sheep as in hoggs.

Nematodirus filicollis is one of the first species to appear in lambs, and the numbers increase to a peak in the following spring; this is followed by a rapid decrease to very low levels of infestation.

The hookworm of sheep, *Bunostomum trigonocephalum*, is a species with a high incidence in Scottish hill sheep and is probably of economic importance. It is interesting, that infestations seem to be acquired slowly by young sheep, that the average burden shows little fluctuation throughout the year, and that no decrease in numbers occurs with age.

The eggs of *Strongyloides papillosus* are usually the first to appear in the faeces of lambs, but throughout the life of sheep on the hill infestations always remain at a low level.

The infestations of *Chabertia ovina* are sometimes heavy in hill sheep, but in general are rather erratic; although there may be a tendency for a spring rise in the hoggs, no clear cut period when peak numbers are present has been noticed.

The numbers of *Oesophagostomum venulosum* reach their peak in the spring in hoggs; at other times of the year and in older sheep heavy infestations occur only occasionally.

Throughout the year some developing young stages are usually present in the lumen of the abomasa and less frequently in the lumen of the small intestines of all ages of hill sheep. It is in the spring, however, that there is a spectacular increase in numbers. Judged by the investigations in 1949–50, when two ewes were slaughtered at fortnightly intervals, the marked increase in the number of larvae, which were developing in the host, occurred about the middle of March. The number of larvae found in the mucous linings of the abomasum and small intestine were always surprisingly low.

The results obtained from this investigation have an important bearing on the problem of control of helminths in hill sheep by prophylactic dosing, since it has revealed data on the times when Scottish hill sheep are most likely to be carrying infestations of pathological importance.

In the first place it has been shown that all ages of sheep can be important carriers of nematodes from one year to another. It has also been shown that the maximum numbers of worms occur about the time the lambs are born, and that the greatest contamination of the pastures by older sheep occurs then and during the following weeks. Furthermore, the highest worm burdens follow the lean winter period on the hill, when sheep are generally in low condition, have lost weight and are less able to withstand heavy infestations.

The emphasis, therefore, must, other things being equal, be on spring dosing. However, many farmers would object to the risk of dosing pregnant ewes a week or two before lambing, which on Scottish hills starts during the middle of April. Unfortunately, in ewes and gimmers larvae do not in the main establish themselves before mid-March, and in early April a high proportion of the infestations consist of immature worms which are unlikely to be completely removed by the anthelmintics usually employed.

On the other hand, to delay dosing until May, after lambing, involves practical difficulties, and, on many farms, would involve risk of injury and mismothering to young lambs at the handling.

It is clear, therefore, that before the results of this investigation can be made the basis of a programme of prophylactic dosing, field trials are necessary in order to find out what risks are involved in spring dosing, what the practical difficulties are, and whether the results are likely to be economically justified. Such trials have already been commenced in Ettrick, and in 1949–50 gave encouraging results.

Finally it should be pointed out that the results of this investigation refer only to Scottish hill sheep and not to sheep on marginal and low ground farms, although these sheep may show a similar seasonal pattern in their worm burden. The earlier time of lambing and the higher level of nutrition of low ground sheep may influence the onset, the general level and the character of the spring rise; until this is fully investigated the most profitable time for dosing is in doubt. Therefore, at present, the practice of dosing ewes early in the year without regard to the type of sheep involved may well bring a possible fruitful method of helminth control to undeserved disrepute.

Whether autumn dosing in addition to spring dosing of Scottish hill ewes is justified has also to be investigated. It should have the advantage of removing those species, such as *Bunostomum trigonocephalum* and *Trichostrongylus axei*, which persist throughout the year, and save the ewes supporting these parasites, when they themselves are on a starvation diet, and it should reduce contamination of the grazings.

Autumn and winter dosing of Scottish hill hoggs is much more likely to be justified. Some species of worms do not fall in numbers in hoggs during the winter, to the extent that they do in ewes, moreover they acquire heavy infestations earlier in the year. Preliminary experiments on hoggs on in-bye fields in Ettrick involving dosing during the autumn, winter and spring are showing encouraging results and have resulted in increases in the weights of the dosed hoggs.

Summary.

1. The investigation shows that the spring increase in worm egg output of Scottish hill sheep is correlated with an increase in worm burden.

2. The increase is mainly due to members of the Trichostrongylidae, and of these the stomach worms, *Ostertagia* spp. and *Trichostrongylus axei*, are the most important.

3. This increase in the worm burden in the spring is due to larvae picked up at that time, and there was no evidence that any were lying dormant throughout the winter in the mucous membrane of the abomasum and small intestine.

4. Some other species, such as *Bunostomum trigonocephalum*, do not show this increase.

5. About January, when infestations in gimmers and ewes are very low, appreciable infestations are common in hoggs.

6. This investigation has revealed the times when the important species of nematodes are present in maximum numbers in Scottish hill sheep ; it is hoped that this data will provide a basis upon which plans for prophylactic dosing can be made.

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Studies on Human Onchocerciasis and *Simulium* in Nyanza Province, Kenya.

II. The Disappearance of *S. neavei* from a Bush-Cleared Focus.

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In a previous paper the writer (1949) reported on the results of a survey of the incidence and distribution of onchocerciasis in Africans in Nyanza Province. The survey was part of an investigation whose main objective was to explore the possibilities of controlling the disease by collecting data concerning the infection and its transmission. Moreover, as the matter was at that time deemed to be one of considerable urgency, it was desirable that control measures, if and when initiated, should be quickly effective and uncomplicated in operation. With this end in view particular attention was directed to the insect vector, *Simulium neavei*, whose then unknown breeding habit appeared to offer the most fruitful line of investigation. Prolonged search, however, both in the field and in the laboratory, failed to throw any light on this obscure problem, whose elucidation is now well known from the account given by van Someren and McMahon (1950); and since anti-larval control measures therefore seemed to be ruled out of consideration, for the time being at any rate, attention was turned to the adult flies.

The restricted flight range of *S. neavei* observed by McMahon (1940) and its confinement to more or less well-defined foci (Buckley, 1949) suggested that they were subject to the influence of certain ecological factors. During the course of the writer's survey when much of the Province was toured for the examination of population samples for onchocerciasis, all water courses were searched for *S. neavei* both in likely and unlikely places, and the experience acquired made it almost possible on arriving at a new locality to recognise it as a habitat of *S. neavei* or exclude it as such before more certain confirmation was made. Empirically it could be stated that a *S. neavei* habitat required (1) a fast-running river or stream, (2) well wooded river banks and (3), a corollary of (1), a hilly or mountainous terrain. *S. neavei* was not found in any locality which lacked one of these three features. It seemed

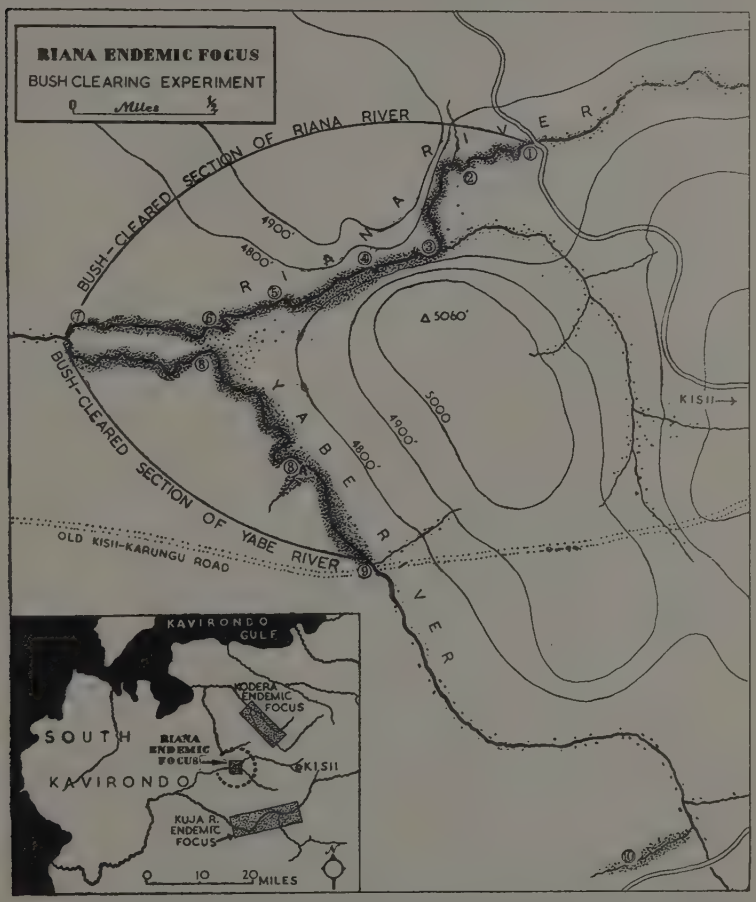
reasonable to assume therefore that together they were essential to the environment of *S. neavei* and that if one of them were removed or perhaps modified the adult flies would be unable to survive. The only practical interference in such an environment which could be then considered was to clear the river banks of vegetation, and accordingly a pilot experiment was carried out on the Chagaroi River at Ngoina, Kericho District, where there was a very dense *S. neavei* population which made it a suitable place for observations on the adult behaviour. From this experiment, evidence emerged that there was a marked reduction in the number of flies caught on human bait in areas which had been completely cleared of undergrowth and partially cleared of trees, as compared with previous routine catches before clearance was begun and with routine control catches made in adjacent uncleared areas at the same time. Although the reduction was not permanent, the evidence that the flies reacted unfavourably to the clearance suggested that if the clearance embraced the whole of a *S. neavei* habitat, the effect on the adult flies might be more far-reaching. It was decided to put this scheme into operation at a small focus in South Kavirondo district, referred to in the previous paper as the " Riana focus ".

Description of the Endemic Focus at Riana

The name Riana refers to a small village and trading centre situated about 10 miles to the west of Kisii, the administrative centre of South Kavirondo District. The Riana River has its origin near Kisii at an altitude of over 5,600 ft. and flows in a westerly direction through hilly and unwooded country to Riana where it has dropped nearly 1,000 ft. during this journey. At Riana the river crosses the Kisii-Homa Bay Road and immediately enters a thickly-wooded stretch with dense undergrowth, about two miles long. The western extremity of this wooded stretch is marked by the confluence of the Yabe River with the Riana River and downstream from this point the vegetation thins out abruptly once again (see Map). The Riana-Yabe stream enters the large Kuja River to the south-west, which flows into Lake Victoria. During its passage through the 2-mile stretch, the Riana River is of moderate size, with an average breadth of about 12 ft., and on it there are four small waterfalls of 3 to 5 ft. in depth. The wooded undergrowth on its banks varies in width considerably from about 10 yards to 80 yards. About the middle of its course it flows between two hills, the steeply-rising Ngeri (5,060 ft.) to the south and a less steep hill to the north (see Map).

The Yabe River is slightly smaller in size than the Riana River.

Its source is in the mountains to the south-east of Riana and at its junction with the old Kisii-Karungu Road it enters a thickly-wooded stretch with dense undergrowth measuring about 1½ miles from this point to the Yabe-Riana confluence. No waterfalls occur in this



stretch, but about half-way along it, the flow is interrupted by rapids.

The area is inhabited by natives of the Kisii tribe and the resident population which is exposed to infection in the focus numbers about 800. Of these, 21.2% were found positive for *O. volvulus* infection.

Agriculture is the main activity and the principal crop is millet. Before the bush-clearing was carried out, the wooded region was populated by a rich avian fauna and amongst the larger mammals were buck, baboon, grey monkey and colobus monkey. It is probable that the latter have now disappeared.

Rainfall data are not obtainable for the area but records are kept regularly at Kisii, some 10 miles away, and the figures for the period 1943–1947, expressed as 4-weekly totals are presented in Graph 4.

Methods of Catching and Estimating Incidence of *S. neavei*

For catching *S. neavei* adults, 3″ by 1″ cork-stoppered glass tubes were used. At the bottom of the tube was a plug of cotton-wool soaked in chloroform and covered with a tightly fitting disc of cardboard. The flies were caught after alighting on human bait, usually a small African boy. The " incidence " of the flies was estimated on the basis of the number of flies caught by a trained collector on one human bait in one hour. If the catching period was only for half an hour, the incidence would be the number of flies caught multiplied by two, and so on.

For the purpose of the bush-clearing experiment it was necessary to establish a standard procedure of fly-catching and incidence estimation which could be carried out over a long period without interruption so that the effects of the clearing on the fly incidence could be observed. Accordingly, fixed spots along the wooded stretches of the two rivers to which *S. neavei* was confined almost exclusively, were set up as catching stations and numbered 1 to 9, seven of which were on the Riana River (see Map and Plate I, A.) and two on the Yabe. Early on, an additional station (No. 8A) was fixed on the Yabe River, making a total of 10 (see Map). During 1945–1947 routine catches were also made on a small wooded tributary of the Yabe River which was left uncleared. (See Map. Catching station No. 10.) The ideal procedure would have been to place ten collectors with bait at each of the ten stations at a fixed time each day and collect flies for one hour. The total number of flies caught, divided by ten, would then be an ideal representation of the incidence on that day. Such a procedure being quite impracticable, as it involved a regular attendance of so many collectors and bait, the following scheme was devised and carried out, with only one accidental interruption in 1944, over a period of 4 and a half years. One collector with human bait was employed throughout who visited each catching station once a week, between the hours of 9.30 a.m. and 11 a.m. Thus, on Monday, he would collect

flies at station No. 1 for half an hour and then move on immediately and collect at No. 2 for half an hour. On Tuesday the same procedure was followed at stations No. 3 and No. 4, and so on. By Friday all ten stations had been visited and the incidence on both rivers was calculated. An example of such a weekly catch is given below.

	Riana River							*Yabe River*		
Catching Station	1	2	3	4	5	6	7	8	8A	9
Flies caught in half an hour ...	1	3	5	12	7	8	4	4	3	1
Estimated incidence	2	6	10	24	14	16	8	8	6	2
Estimated av. incidence per week				11.4				5.3		

For the purpose of illustrating the incidence (Graph 1), the estimated average incidence for 4–weekly periods was taken, otherwise the graph would be too unwieldy.

In order to keep a check on both the efficacy of this method of procedure and on the work of the African collector, " check " catches were made at irregular intervals, by employing ten collectors with bait to catch flies at each station simultaneously on the same day. The results of these catches invariably corresponded with the current trend of the fly incidence estimated by the routine method in use.

The importance of choosing a more or less fixed time during the day for the catching of the adult flies was indicated from observations made at Ngoina, in Kericho District. It was noticed there that the number of flies caught on human bait seemed to reach a peak in the forenoon and again in the afternoon, with a marked reduction in the number caught at midday. This was confirmed on several occasions when systematic all-day catches (from 8 a.m. to 6 p.m.) were carried out and the numbers collected every half an hour or quarter of an hour were tabulated. When these figures were graphed a diphasic curve resulted, which moreover almost invariably showed a higher peak in the afternoon than in the forenoon. (These findings will be described in more detail and discussed in a later paper.)

The Bush-Clearing Experiment.

The logical procedure to obtain quick results from the effect of bush clearing on the fly population would have been to deforest the locality completely and remove all the undergrowth. But for aesthetic as well as practical reasons (erosion, expense and time) the idea of complete deforestation was dismissed from the start. The ideal object

was to discover the *minimum* amount of clearing necessary to reduce the fly population to insignificant numbers or to eradicate it. It was decided therefore to apply a discriminative clearing to the area, consisting of complete removal of the dense undergrowth, employing a gang of men with cutlasses, and the reduction, by burning, of the accumulated masses of vegetation. Together with this a limited number of the smaller trees was cut down. (See Plate I, B).

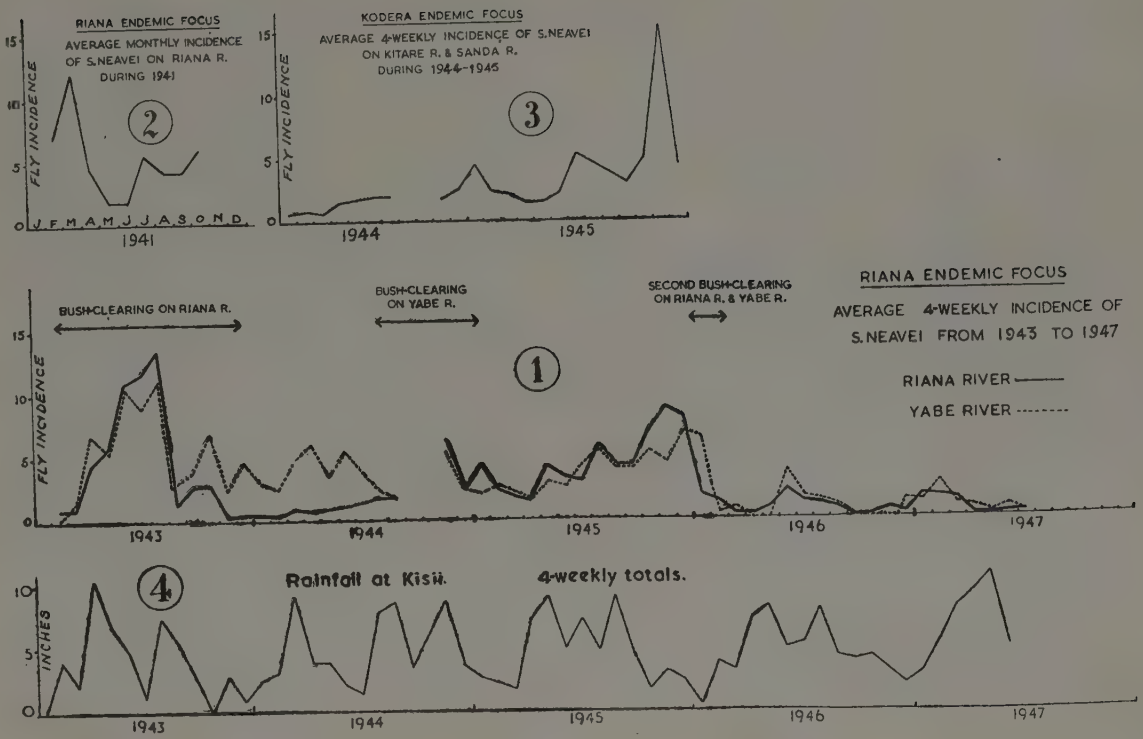
Bush-clearing on these lines was begun about the middle of February, 1943, and applied to the Riana River alone, leaving the Yabe River as a temporary control. By July of that year this programme was completed and the gang of workers was paid off, except for a small number who were retained for maintenance (keeping down regenerating undergrowth) and for burning.

By August, 1943, the *S. neavei* incidence on the Riana River and the Yabe was not showing the difference which was expected as a result of the clearance so further tree-cutting on the Riana River was begun and continued till December, 1943. (This step, which was taken mainly from motives of impatience, was probably unwise as it may have been unnecessary. Subsequent events showed that if the clearance was responsible for the reduction in the fly population which occurred, it took effect very slowly indeed and possibly the February–July system might by itself have been sufficient.)

It will be seen from Graph 1 that there was a steady increase in the *S. neavei* incidence on both the Riana River and the Yabe River during the first period of the bush-clearing (undergrowth and small trees); that around about July–August, 1943, there was a sharp descent in both the incidence curves. After this the two curves, which hitherto had kept fairly close together, began to diverge, the Yabe River curve remaining in the region of 3 to 6 incidence while that of the Riana River descended to less than one. This was the hoped-for result which resembled, on a much larger scale, the pilot experiments at Ngoina, Kericho.

The time now seemed ripe to apply the clearing process to the Yabe River. This, however, could not be started until the middle of 1944, a few months after the writer had left Kenya to carry out a helminthological survey in Northern Rhodesia during the period March–December. During this time the routine collecting of adult flies was unfortunately stopped owing to a misunderstanding, for about 3 months.

A glance at Graph 1 shows what was happening in the second year of the experiment. In the first half, the fly incidence on the Riana River was showing a disturbing tendency to rise. That of the Yabe


RIANA ENDEMIC FOCUS
AVERAGE MONTHLY INCIDENCE OF S.NEAVEI ON RIANA R. DURING 1941
FLY INCIDENCE
J F M A M J J A S O N D
1941
2
KODERA ENDEMIC FOCUS
AVERAGE 4-WEEKLY INCIDENCE OF S.NEAVEI ON KITARE R. & SANDA R. DURING 1944-1945
1944
1945
3
BUSH-CLEARING ON RIANA R.
BUSH-CLEARING ON YABE R.
SECOND BUSH-CLEARING ON RIANA R. & YABE R.
1
RIANA ENDEMIC FOCUS
AVERAGE 4-WEEKLY INCIDENCE OF S.NEAVEI FROM 1943 TO 1947
RIANA RIVER
YABE RIVER
1943
1944
1945
1946
1947
4
Rainfall at Kisii.
4-weekly totals.
INCHES

River, strangely enough, came down to meet it. After that, the cessation of the routine collecting leaves us in ignorance as to the incidence on either river; but when it was begun again, towards the end of the year when the Yabe River clearing was nearly complete, the incidence on both rivers was the same, namely about 5, and showed a downward trend.

Throughout 1945 the two curves kept together and their steady rise during this year gave the impression that the experiment was a failure. But again the unexpected thing happened. There was a sudden drop in the incidence at the turn of the year, a rather feeble recrudescence in the middle of 1946 followed by another early in 1947, after which the incidence fell to insignificant numbers and the routine collecting was abandoned. Subsequent "check" catches in the area in 1948 and 1949 revealed that *S. neavei* was no longer present. (The second bush-clearing indicated in Graph 1 was merely a clearing up of undergrowth which had sprung up during 1945.)

Discussion.

From the scientific aspect the design of this experiment was far from perfect. For example, it would have been desirable to have carried out routine collecting at the ten catching stations for 6 or 12 months *before* the bush-clearing was begun, to find out what was the normal incidence of *S. neavei* in the area. Here again, the time factor must be held responsible, for the need for quick results took precedence. The only available data on this score was from some relatively unmethodical collecting done in 1941, from February to October. These catches were made at no fixed time of the day, as the diphasic biting habit of *S. neavei* was not then properly realised, nor at the fixed catching stations established later for the clearing experiment. (The importance of these was borne out subsequently by the fact that some catching stations consistently yielded more flies than others.) However, as the duration of each catch was always noted down, it was possible to estimate the incidence, as defined earlier. The average monthly incidences are shown in Graph 2. The resemblance between this curve and that of Graph 1 is noteworthy and suggests a seasonal variation of the incidence.

It would also have been desirable to have a control for the Riana experiment throughout its progress at some other endemic focus. This was not done until 1944, when ten catching stations were fixed on the Kitare and Sanda Rivers at the Kodera endemic focus, and an African collector was detailed to this area. Unfortunately the data obtained cannot be regarded as absolutely reliable, as the work there could not

be given enough supervision. It was continued for two years only (Graph 3) as this focus was then taken over for the D.D.T. experiment of Garnham and McMahon (1947) which proved so successful.

Whatever the defects of the Riana experiment, it must be admitted that the result aimed at was ultimately achieved, and it seems reasonable to conclude that the disappearance of *S. neavei* was in fact brought about by the alteration to its adult habitat, although the minimum amount of clearance which might have been necessary was not ascertained. Against this conclusion is the puzzling recrudescence of the incidence in 1945 after the Yabe River had been cleared, when the logical expectation was quite the reverse of what actually happened. However, even assuming that the bush-clearing was in fact responsible for eradicating the flies, this method of control is of limited value in that it is practicable only in small foci such as Riana. Moreover, it is now superseded by the less expensive and more rapidly effective method of larval control discovered by Garnham and McMahon.

It could be claimed in its favour perhaps, that it is more permanent than D.D.T. eradication, for it is extremely unlikely that in the event of the accidental re-introduction of *S. neavei* into the Riana area, the flies would be able to become established there again, whereas this remains a theoretical possibility in a D.D.T. treated focus.

Summary.

1. An experiment in bush-clearing was carried out at a small endemic focus of onchocerciasis in South Kavirondo district with a view to observing its effect on the adult population of *S. neavei*.

2. The clearing consisted of the complete removal of undergrowth and partial removal of trees, from the banks of fly-infested portions of two confluent rivers, the Riana and the Yabe.

3. In the first year of the experiment the clearing was carried out on the infested section of the Riana River, after which there was a decrease in the incidence of *S. neavei* on this section compared with that on the uncleared Yabe River.

4. In the second year a similar clearing was effected on the infested section of the Yabe River, after which the *S. neavei* incidence on both sections came together again and during the third year showed a steady increase.

5. In the fourth year the *S. neavei* incidence on both sections fell suddenly to a very low figure, revived slightly half-way through this year and fell again.

6. Early in the fifth year there was another slight revival followed by a reduction from which the fly population never recovered, as subsequent searches in the locality in the next two years failed to reveal a single adult *S. neavei*.

7. It is concluded that the alteration, by bush-clearing, of the natural environment of the adult *S. neavei* was responsible for the extinction of the flies.

ACKNOWLEDGMENTS.

For the execution and completion of the long-drawn-out experiment described above, the writer was dependent upon the willing co-operation of members of the Division of Insect-borne Diseases and the Health Office of the Colony. He is especially grateful to Mr. W. E. Grainger who supervised the routine collecting of flies over a period of nearly three years, after the writer had left Kenya Colony, and for the regular transmission of these records to London ; to Mr. J. P. McMahon, who carried out the important " check " investigations in 1948 and 1949 which showed that *S. neavei* had disappeared from the Riana endemic focus ; and to Mr. A. T. Matson, Health Inspector at Kisii, for his efficient organisation during the writer's absence, of the bush-clearing on the Yabe River in 1944 and again in 1946. The constant encouragement and helpful advice of Mr. C. B. Symes in all matters entomological is gratefully acknowledged ; and also that of my colleague Dr. P. C. C. Garnham who was then in charge of the Division of Insect-borne Diseases.

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Plate I,A.—Part of Riana River before clearing, showing position of fly-catching stations numbers **2** to **7**. B. The same after clearing ; slightly closer view.

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Further Observations on the Distribution of the Beet Eelworm.

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Field surveys to determine the distribution of beet eelworm (*Heterodera schachtii* Schm.) in the important beet growing areas of East Anglia were begun in 1937. The results of this work up to the end of 1943 have already been published (Petherbridge and Jones 1944). Surveys were continued in 1944 and then less extensively until the dissolution of the old Advisory Service in 1946. In 1948, a new type of survey was attempted through the field staffs of the British Sugar Corporation, Ltd., and continued in 1949 and 1950.

The methods used in field-to-field surveys have already been described (Petherbridge and Jones 1944). In the factory eelworm surveys, each fieldman examined ten beet fields in the period June-July. Wherever possible, the fields chosen lay in localities where frequent cropping with beet, mangolds or *Brassicae*, gave reason to suppose that eelworm might occur. The selection of the fields was left to the discretion of the fieldmen, except that in the Fenland area attention was directed to fields outside localities known to be heavily infected. Fields were first examined for poor patches in the crop and, if any were found, plants were lifted and the roots searched carefully for eelworm cysts. Next, twenty plants were lifted where the crop appeared healthy and the roots searched as before. At least ten of these plants were taken from the region of the gateway, for, if any part of the field is infected, the infection soon establishes itself here because of the constant passage of carts and implements. The remaining ten plants were taken from other parts of the field, especially any parts that might have been cropped more frequently with beet or mangolds. Where no definite gateway occurred, special attention was paid to areas adjoining the obvious traffic routes. Altogether, 150 fieldmen participated in the survey and some 1,500 fields were examined in each of the years 1948, 1949 and 1950. At each factory, at least two members of the field staff have had an opportunity of examining beet eelworm cysts in the laboratory and all members have seen cysts on infected plants.

The results obtained from these factory surveys are summarised in Table 1.

TABLE 1.

Summary of results from field surveys by factory fieldmen.

	1948	*1949*	*1950*	*Totals*
Number of fields examined	1560	1500	1510	4570
Number infected	9	5	10	24
Number of fields in old localities ..	4	1	4	9
Number of fields in new localities ..	5	4	6	15

TABLE 2.

Additional localities in which beet eelworm has been found.

1943–1950

Year	*County*	*Locality*	*Authority*
1944	Bedfordshire	*Ampthill	B. D. W. Morley
	Berkshire	*Abingdon	,, ,,
	Buckinghamshire	Aylesbury	,, ,,
		*Buckingham	,, ,,
	Hertfordshire	Harpenden	,, ,,
	Norfolk	*Thetford	,, ,,
	Rutland	*Uppingham	,, ,,
	Yorkshire	*Ripon	H. W. Thompson
1945	Northamptonshire	*Irchester	B. D. W. Morley
	West Suffolk	*Newmarket	,, ,,
1947	Lincolnshire	Barkston	
1948	East Suffolk	Melton	
		Sproughton	
		Wherstead	
1949	Cambridgeshire	Haddenham	N.A.A.S.
	Lincolnshire	**Brigg	
	Yorkshire	Wistow	
	West Suffolk	Lakenheath Station	N.A.A.S.
		Lakenheath Village	
1950	Cambridgeshire	Waterbeach	K. P. Humphries
	Lincolnshire	*Marston	
		*Walpole St. Andrew	
	Norfolk	Cantley	
		Horsford	
	West Suffolk	West Row	
	Hampshire	*Basingstoke	

* Sewage farms.

** The waste soil dump of the Brigg Beet Sugar Factory.

Where no authority is given the infection was confirmed by the writer and passed on to the National Agricultural Advisory Service.

Fig. 1.—The distribution of beet fields examined during the survey by factory fieldmen in 1948, 1949 and 1950 ; 4,340 fields in England and Wales and a further 230 fields in Scotland around the Cupar Beet Sugar Factory, Fife (not shown).

Each dot represents one field.

The area covered by the factory surveys is indicated in Fig. 1. Table 2 gives a list of new localities outside the scheduled areas where beet eelworm has been found in the period 1944–50, while Fig. 2 shows the distribution of beet eelworm in England and Wales, so far as it is known at present. Beet eelworm has not yet been found in Scotland.

Scattered beet eelworm infections occur in eastern England from south Yorkshire to the Thames (Fig. 2). Outlying infections further south and west in areas less extensively searched, suggest that the eelworm may be more generally dispersed. Of the eighteen beet-sugar factories and the Southern Area based on Salisbury, only the factories at Bardney, Lincs ; Cupar, Fife ; and Kidderminster, Worcs ; are without known eelworm infections within their operational zones. Twenty-one of the isolated infections occur on sewage farms employing land irrigation for the purification of the effluent, but most of the infected farms are small and many lie outside the main beet-growing areas. The sewage farm near Norwich, however, where more than 100 acres of land are infected, constitutes a menace to important beet-growing areas in Norfolk. Four of the isolated infections are waste soil dumps at beet sugar factories (Brigg, Ely, King's Lynn and Wissington). In fact. with the possible exception of the Cupar factory in Fife, the waste soil dumps of all factories may be regarded as infected, since most factories have infected land within their operational zones. Outside the Fenland areas scheduled under the Sugar Beet Eelworm Order in 1943 and 1944, field infections of some importance occur at Cantley, Norwich ; Haxey, Isle of Axholme ; Lakenheath and West Row, West Suffolk ; South Cave, Yorkshire ; Sproughton, East Suffolk ; and Waterbeach, Cambridgeshire.

The results of the survey by factory fieldmen indicate that, outside the heavily infected areas in the Fens, only 24, or 0·5%, of the 4,570 fields examined were infected. This result, however, must be qualified. Infected fields may be placed in several arbitary categories :—

Category 1. " Beet-sick," extensive patches of stunted plants.

2. Scattered patches of stunted plants.

3. No patches apparent but eelworm cysts generally distributed and numerous.

4. As above but cysts present in small numbers only.

5. Eelworms cysts on plants at a few points in the field.

6. Occasional cysts on isolated plants.

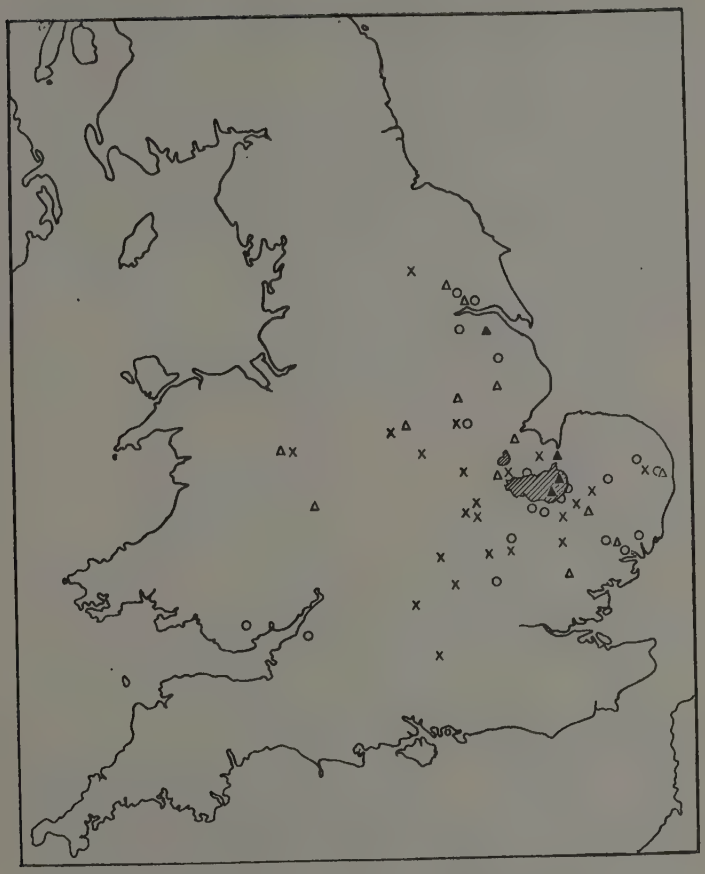

Fig. 2.—The known distribution of beet eelworm in England and Wales. O, isolated infected areas; X, infected sewage farms; Δ, Beet Sugar Factories; ▲, Beet Sugar Factories with infected waste soil dumps; shaded area, the large Fenland areas scheduled under the Beet Eelworm Order 1943.

The detection of " beet-sick " fields presents no difficulties, nor is there any difficulty in detecting eelworms in apparently healthy crops where the eelworm cysts are fairly numerous. With semi-trained observers of varying capabilities, only fields of these types (categories 1–3) can be detected with any degree of certainty. Even with fully trained observers, the detection of fields in categories 5 and 6 is still subject to a considerable measure of statistical uncertainty. Thus, at the limit, if one cyst occurs on one plant in a ten acre field with 25,000 plants per acre, then by lifting twenty plants there are 12,499 chances to 1 against the infection being detected, assuming that the entire root systems of all twenty plants are lifted intact and the single cyst is observed. In practice, only about one-tenth of the root system of a plant is lifted and this portion must bear several cysts (say 5) before detection can be assured. On this basis, an infection cannot be detected before one of the plants in the field bears about 50 cysts, and the chances against detection remain as before, 12,499 to 1. The non-random distribution of cysts in the early stages of infection, would tend to increase the chances of detection if it led to the appearance of crop symptoms or to the presence of concentrations of cysts near gateways and other places more intensively searched.

Enough has been said to illustrate the limitations of field survey, nevertheless, it compares favourably with survey by soil sampling. Here, setting aside the difficulties of identification of lemon-shaped cysts, it is rarely possible to search a representative sample of soil greater than 200 g. in weight. In field surveys, each plant lifted effectively " searches " some 500 g. of soil and, in addition, an indication is given of the performance of the host crop in the affected field.

The results of the field survey by factory fieldmen may now be re-expressed as follows : " Out of 4,570 fields examined only 24, or 0·5%, were sufficiently heavily infected with beet eelworm (categories 1–3) for the eelworm to be apparent," or alternatively " only in 24, or 0·5%, of the fields was the eelworm population above or near the economic zero." (The economic zero may be defined as that population level above which serious crop injury appears.) As an expression of the present status of the beet eelworm problem outside the Fenland area, these statements are probably near the truth. Interest, however, centres around fields in categories 4, 5 and 6, as these give a better indication of the possible magnitude of future problems. If no intensive field surveys with trained observers had been undertaken in the Fenland area of East Anglia and only the reports of farmers,

factory fieldmen or county staffs had been available, the magnitude of the beet eelworm problem in the Fens would have remained unassessed. Three examples illustrate this point.

(1) In 1935, a fenland farmer sought advice because his beet yields had fallen to 3–4 tons an acre on certain fields, despite the high state of fertility in which the land was maintained. Examination of the farm showed that nearly every field was infected with eelworm. Examinations of neighbouring farms revealed additional " beet-sick " fields. Surveys between 1937 and 1945 showed that the infected area comprised an almost continuous block of fields covering more than one square mile, in which the only uninfected land had been down to grass or had grown susceptible crops at infrequent intervals. Many isolated infected fields were also found around the edges of the continuous area. Of all these infected fields, not more than three (category 1) were reported by beet sugar factories and only one (category 3) by the existing county staff.

(2) In 1935, Wissington beet-sugar factory sent in samples of " beet-sick " plants from Larman's Fen, Norfolk. In the following year, Mr. Petherbridge, working in conjunction with the factory, found several more fields exhibiting signs of " sickness ". Surveys in subsequent years have shown that at least 26 fields are infected.

(3) In 1942, the County Organiser for the Isle of Ely drew attention to a " beet-sick " field (category 1) in the Manea area. Two years' intensive search brought to light 41 more infected fields (categories 3–6) and one more " beet-sick " field (category 2).

Many similar examples could be given. In general, where a single heavily infected field is found in an intensive beet-growing area, at least 10 more are detected in due course if a careful search is made by trained personnel.

Perhaps the greatest value of the survey by factory fieldmen is that it has served to focus attention on new areas where beet eelworm has not previously been suspected. Further investigation of these areas is desirable to assess the limits of infection.

Thanks are due to the field staff of the British Sugar Corporation Ltd., for undertaking the examination of fields in 1948, 1949 and 1950, and also to Mr. K. P. Humphries for assistance with samples and correspondence arising from the factory surveys. Mr. B. D. W. Morley assisted in field surveys in 1944 and 1945.

SUMMARY.

An account is given of further observations on the distribution of the beet eelworm (*Heterodera schachtii* Schm.) in the period 1944–1950. Scattered infections occur in eastern England from South Yorkshire to the Thames and outlying infections suggest that the eelworm may be more generally dispersed. As yet, there are no records of beet eelworm occurring in Scotland. The limitations of the method of field survey are briefly discussed.

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